

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)

0342/1D516-US1

09/601965

INTERNATIONAL APPLICATION NO.
PCT/US99/02940INTERNATIONAL FILING DATE
08 February 1999PRIORITY DATE CLAIMED
09 February 1998

TITLE OF INVENTION

A NOVEL FUNGAL PROTEIN CRITICAL FOR EXPRESSION OF FUNGAL PROTEINS

424 Rec'd PCT/PTO 09 AUG 2000

APPLICANT(S) FOR DO/EO/US

Craig M. THOMPSON, Fan LONG, and Richard C. WOBBE

Applicant herewith submits to the United States Designated/Elected office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S. C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371 (f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S. C. 371 (b) and PCT Articles 22 and 39 (1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S. C. 371 (c) (2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. A translation of the International Application into English (35 U.S. C. 371 (c)2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c) (3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unsigned).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98 (with references).
12. ☐ An assignment document for recording. A **separate** cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney an/or address letter.
16. ☒ Other items or information: **Sequence listing: diskette and paper copy; Statement Pursuant to 37 C.F.R. 1.821; Letter Accompanying U.S. National Phase Filing and copy of the International Preliminary Examination Report**

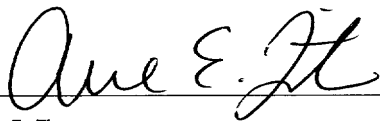
EXPRESS MAIL CERTIFICATE

Date 8/9/00 Label No. 2628221992

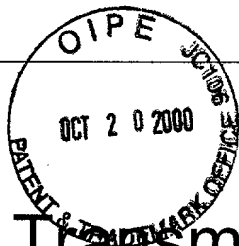
I hereby certify that, on the date indicated above I deposited this paper or fee with the U.S. Postal Service & that it was addressed for delivery to the Commissioner of Patents & Trademarks, Washington D.C. 20531 by "Express Mail Post Office to Addressee" service.

Name (Print)

Signature

U.S. APPLICATION NO. (if known sec 37 C.F.R.1.50) 09/601965		INTERNATIONAL APPLICATION NO. (if known sec 37 C.F.R.1.50) 534 Rec'd PCT/PTO		<div style="text-align: right;">09 AUG 2008</div> Patent & Trademark Office Fee & Deposit Account Number 0342/1D516-US2	
17. <input checked="" type="checkbox"/> The following fees are submitted:				ALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492 (a)(1)-(5)): Search Report has been prepared by the EPO <input type="checkbox"/> or JPO <input type="checkbox"/> \$840.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00					
No international preliminary examination fee paid to USPTO(37 CFR 4.482) but international search fee paid to USPTO (37 CFR 1.445 (a) (2))... \$690.00					
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$970.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) ... XXX \$96.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$96.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	13-20	0	0 X \$18.00	\$	
Independent Claims	6-3	3	3 X \$78.00	\$234.00	
Multiple dependent claims(s) (if applicable) + 260				\$	
TOTAL OF ABOVE CALCULATIONS =				\$330.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later the <input type="checkbox"/> 20 <input type="checkbox"/> 39 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
+					
TOTAL NATIONAL FEE =				\$330.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). the assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$	
+					
TOTAL FEES ENCLOSED =				\$330.00	
				Amount to be refunded	\$
				charged.	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$330.00 to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No.04-0100 in the amount of \$ to cover the above fees. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 04-0100. A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO Anne E. Zitron Darby & Darby P.C. 805 Third Avenue New York, New York 10022-7513					
			SIGNATURE		
			NAME	Anne E. Zitron	
			REGISTRATION NO.	41,391	

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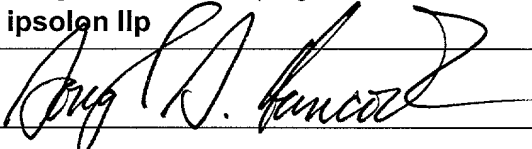
Transmittal Letter

Application Number:	09/647683
Filing Date:	September 28, 2000
First Named Inventor:	McHenry
Attorney Docket Number:	1201-009/ddh
Title	<i>Folding Pocket Knife With Lock</i>

Enclosed for filing in the above referenced application are the following:

- ☒ Petition to Make Special (37 C.F.R. § 1.102(d))
- ☒ Petition Fee of \$130.00 (check number 2994)
- ☒ Other: return receipt postcard

The Commissioner is hereby authorized to charge any additional fees that may be required in connection with prosecution of this application to Deposit Account No. 50-0241

Firm or Individual Name:	Douglas D. Hancock (Registration No. 35,889) ipsolen llp
Signature	
Date	October 20, 2000

Express Mail No.: EL304655642US

Customer Number: 21,034

Express Mail Certificate

Date: 8/9/00 Label: 628221992

534 Rec'd PCT/PTC 09 AUG2000

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DB Peck & Peck [Signature]
Name (Print) Signature

Docket No.: 0342/1D516US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Craig M. THOMPSON et al.

Serial No.: TBA Art Unit: TBA
(National Phase of PCT/US99/02940)

Filed: Concurrently Examiner: TBA

For: NOVEL FUNGAL PROTEIN CRITICAL FOR EXPRESSION OF FUNGAL PROTEINS

**PRELIMINARY AMENDMENT,
SUBMISSION OF SEQUENCE LISTING
AND
STATEMENT PURSUANT TO 37 C.F.R. § 1.821**

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Please amend the above-identified patent application as follows:

In the Specification:

After page 45 and before the claims, please insert the attached paper copy of the Sequence Listing.

REMARKS

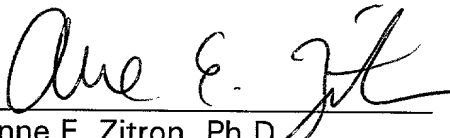
In compliance with 37 C.F.R. § 1.821, applicants herein provide a Paper Copy of a Sequence Listing as well as a Computer Readable Form (diskette).

In addition, applicants herein provide an amendment to the specification directing the entry of the paper copy of the Sequence Listing into the specification after page 45 and before the claims.

Finally, pursuant to Rule 821(f), applicants herein state that the content of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labeled diskette, specifically the ASCII-encoded file therein labeled "seqlist.txt", are identical.

Consideration of this Amendment and submission of the enclosed diskette and paper copy are respectfully requested.

Respectfully submitted,



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**A NOVEL FUNGAL PROTEIN CRITICAL FOR
EXPRESSION OF FUNGAL PROTEINS**

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This application claims priority under 35 U.S.C. § 119 from provisional patent application Serial No. 60/074,100, filed February 9, 1998, the entire disclosure of which is incorporated by reference herein in its entirety.

20

Field of the Invention

This invention pertains to proteins required for activated transcription in yeast and fungi, nucleic acids encoding these proteins, and methods of using these proteins.

25

Background of the Invention

30

Most fungi are opportunistic pathogens, producing serious disease only in compromised individuals. As the result of an aging population and an increase in the number of immunocompromised patients, specifically patients with acquired immunodeficiency syndrome (AIDS), patients undergoing cancer and corticosteroid therapy, as well as in patients undergoing organ transplantation, fungal infections are increasing rapidly.

Most infections begin by colonization of either the skin, a mucosal membrane, or the respiratory epithelium. Passage through the initial surface barrier is accomplished through a mechanical break in the epithelium or enzymatic degradation

5 or spore dissemination. Most fungi are readily killed by neutrophils and are only opportunists, but some species are resistant to phagocytic killing and may infect otherwise healthy individuals.

Fungi parasitize many different tissues. Superficial fungi cause indolent lesions of the skin. Subcutaneous pathogens cause infection through the skin and spread by subcutaneous or lymphatic routes. Opportunistic fungi such as *Aspergillus* are widespread in the environment and are present in normal flora and cause disease mostly in immunocompromised individuals. Systemic fungi are the most virulent and may cause progressive disease leading to deep seated visceral infections in otherwise healthy individuals (see e.g. *Sherris Medical Microbiology, Third Edition*, Kenneth J. Ryan, ed., Appleton & Lange, Norwalk, CT, 1994).

The major fungal pathogens in North America are *Histoplasma capsulatum*, *Coccidioides immitis*, *Blastomyces dermatitides*, *Cryptococcus neoformans*, *Candida* species and *Aspergillus* species (*Medically Important Fungi, Second Edition*, Davise H. Larone, Ed., American Society for Microbiology, Washington, D.C.). *Histoplasma capsulatum* causes histoplasmosis, which may be chronic or progressive and fatal. It is either a localized or disseminated infection, primarily of the reticuloendothelial system. *Coccidioides immitis* causes coccidioidomycosis, a highly infectious disease that is endemic to the southwestern United States and may be a chronic, sometimes fatal infection involving the skin, bone, joints, lymph nodes, adrenal glands and central nervous system. *Blastomyces dermatitides* causes blastomycosis, a chronic infection characterized by suppurative and granulomatous lesions that begins in the lungs and is disseminated to the skin and bones. *Cryptococcus neoformans* causes cryptococcoses, which may be a chronic infection involving the central nervous system. *Candida albicans* is the most frequent cause of candidiasis, which ranges from an acute to a chronic infection involving any part of the body. *Aspergillus fumigatus* is one of the most frequent causes of aspergillosis, which is an opportunistic infection in immunosuppressed individuals.

Fungi are a distinct class of microorganism, most of which are free-

5 living. They are eukaryotic organisms containing a nuclear membrane, mitochondria and an endoplasmic reticulum. The cell structure includes a rigid cell wall of mannan, glucan, and chitin and a cytoplasmic membrane with a large percentage of ergosterol. The size and morphology of fungi vary. There are monomorphic yeasts and yeast-like organisms including *Candida*, *Cryptococcus*, and *Saccharomyces*. There are
10 monomorphic molds, such as *Aspergillus* and *Coccidioides*. Some thermally dimorphic fungi, such as *Blastomyces dermatitides* and *Histoplasma capsulatum*, grow either in a yeast or mold phase.

Only a handful of agents are active against fungi. For life threatening disease caused by any of these fungi, amphotericin B is the agent of choice. This
15 drug, however, is associated with numerous severe side effects such as fever, dyspnea and tachycardia, and dosage is limited over the lifetime of the patient because of renal toxicity. An agent frequently used concurrently is flucytosine, a nucleoside analog that cannot be used independent of other agents because of the rapid appearance of resistance. Untoward effects of treatment with flucytosine include leukopenia,
20 thrombocytopenia, rash, nausea, vomiting, diarrhea, and severe enterocolitis.

In conditions where the patient's life is not threatened, ketoconazole can be used as a long-term therapy for blastomycosis, histoplasmosis, or coccidioidomycosis. Fluconazole also has a significant role in the treatment of superficial fungal infections. Both compounds are from the same class, the triazoles,
25 and are cytostatic. The emergence of resistance and hepatic toxicity limit the use of triazoles such as fluconazole and ketoconazole. The newest triazole, itraconazole, has similar pharmacokinetics and spectrum of activity as fluconazole. None of the azoles can be used for life threatening or deep seated fungal infections, but they are effective in reducing colonization of fungi such as *Candida* and for treating superficial
30 mycoses.

All major antifungal agents attack directly or indirectly a component of the cell wall--ergosterol. Amphotericin B and other polyene macrolides interact with ergosterol in the cell membrane and form pores or channels that increase the

5 permeability of the membrane. Resistant to amphotericin B in mutant strains is accompanied by decreased concentrations of ergosterol in their cell membranes. Imidazoles and triazoles inhibit sterol 14- α -demethylase, a microsomal cytochrome P₄₅₀-dependent enzyme system. Imidazoles and triazoles thus impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14- α -
10 methyl sterols, which impair certain membrane-bound enzyme systems (See e.g. *The Pharmacological Basis of Therapeutics, Eighth Edition*, Goodman and Gilman, Pergamon Press, 1990).

Development of an effective method and composition for treatment of fungal infections is a critical goal of the pharmaceutical industry. The pharmaceutical
15 industry has made numerous efforts to identify fungal-specific drugs, with only limited success to date. It would be of great value to identify a new class of antifungal drug that blocks a fungal target other than ergosterol. This target should be fungal-specific and should lead to development of a drug that is effective against the organisms that are resistant to current therapy.

20 Drug development often relies on the screening of a large number of potential inhibitors before a specific lead compound inhibitor is found. Assays developed for such screens are complex and must mimic the physiological activity of the target protein. Thus, it is critical for the development of these screens to define the proteins involved in the targeted process and to have discovered a means of purifying
25 the necessary components of the assay for use in the assay. In addition, it is useful to have clones for the protein components of the assay to facilitate the production of the components.

Therefore, there is a need in the art to identify one or more fungal constituents, preferably polypeptides, that can serve as useful targets for drug
30 intervention, and for methods and compositions for identifying useful anti-fungal agents and treating fungal infections.

5 Summary of the Invention

The present invention provides an isolated fungal polypeptide, termed TAF-145, that is necessary for activated transcription of particular genes (i.e., gene-specific transcription) in *Candida albicans*. The invention also includes nucleic acid sequences encoding TAF-145, as well as DNA vectors and transformed cells suitable for recombinant expression of this polypeptide. The DNA sequence of *C. albicans* TAF-145 (SEQ ID NO:1) is set forth in Figures 3A-3G.

In one aspect, the present invention provides methods and compositions for inhibiting gene-specific transcription in *C. albicans*, comprising contacting the cell with an agent that selectively interferes with the transcriptional activation activity of the *Candida* TAF complex, preferably with the activity of TAF-145. In one embodiment, the inhibitory agent is a fragment of TAF-145 that inhibits TBP-TAF interaction.

In another aspect, the invention provides a method for high-throughput screening of large numbers of test compounds to identify an agent useful in the treatment of fungal diseases, specifically those caused by *C. albicans*. The method is carried out by exposing the TAF complex or TAF-145 to TATA Box Binding Protein (TBP) in the presence of at least one test compound, followed by determining that the compound inhibits the binding of TBP to the TAF complex or TAF-145. In another embodiment, candidate antifungal agents are identified as those that bind directly to TAF-145, which are identified using methods such as, e.g., those disclosed in U.S. Patent 5,585,277. In yet another embodiment, candidate antifungal agents are identified as those that inhibit the histone acetyl transferase (HAT) activity of TAF-145.

These and other aspects of the present invention will be apparent to those of ordinary skill in the art in light of the present description, claims and drawings.

5 **Brief Description of the Drawings**

Figures 1A-1C together illustrate a protein sequence comparison among TAF-145 derived from *Saccharomyces cerevisiae* (SEQ ID NO:3), *S. pombe* (SEQ ID NO:4), *Drosophila* (SEQ ID NO:5), and humans (SEQ ID NO:6).

Figure 2A is a schematic illustration of the cloning strategy used to
10 obtain the sequence of *C. albicans* TAF-145 (SEQ ID NO:1) using degenerate oligonucleotide PCR.

Figure 2B is a photographic illustration of an agarose gel in which TAF-145-specific PCR products are displayed.

Figures 3A-3G together depict an illustration of the entire nucleotide
15 sequence of the *C. albicans* TAF-145 gene (SEQ ID NO:1) and the predicted amino acid sequence (SEQ ID NO:2).

Figures 4A-4D together illustrate a protein sequence comparison between *C. albicans* (SEQ ID NO:2) and *S. cerevisiae* (SEQ ID NO:3) TAF-145.

Figures 5A-5C together illustrate a protein sequence comparison
20 among the TAF-145 proteins from three yeast species: *C. albicans* (SEQ ID NO:2), *S. cerevisiae* (SEQ ID NO:3), and *S. pombe* (SEQ ID NO:4).

Figures 6A-6C together illustrate a sequence comparison among the TAF-145 homologs from *C. albicans* (SEQ ID NO:2), *S. cerevisiae* (SEQ ID NO:3), *S. pombe* (SEQ ID NO:4), *Drosophila* (SEQ ID NO:5), and human (SEQ ID NO:6).

Figure 7A is an photographic illustration of the results of an
25 experiment in which a *S. cerevisiae* strain temperature sensitive for TAF-145 was transformed with DNA encoding either *C. albicans* TAF-145 or *S. cerevisiae* TAF-145. Only the *Saccharomyces*-derived sequence was able to support growth of the strains under restrictive conditions.

Figure 7B is an photographic illustration of the results of an experi-
30 ment in which a *S. cerevisiae* strain deleted for TAF-145 was transformed with DNA encoding *C. albicans* TAF-145 or *S. cerevisiae* TAF-145. Only the *Saccharomyces*-derived sequence was able to support growth of strains under restrictive conditions.

5 Figures 8A and 8B together illustrate a sequence comparison among the histone acetyltransferase (HAT) domains from *C. albicans* (SEQ ID NO:7), *S. cerevisiae* (SEQ ID NO:8), *S. pombe* (SEQ ID NO:9) and human (SEQ ID NO:10).

 Figure 9 is a photographic illustration of an SDS-PAGE gel of extracts from uninduced (U) and induced (I) cells following Ni-NTA chromatography.

10 Figure 10A is an illustration describing the electroelution of TAF proteins from an SDS-PAGE gel.

 Figure 10B is a photographic illustration of the purity of the electroeluted proteins as determined by a coomassie stained 12 % SDS-PAGE gel.

15 Figure 11 is a graph depicting the results of the ELISA for testing the specificity of rabbit sera against the recombinant CaTAF145 HAT domain.

 Figure 12 is a photographic illustration of a Coomassie stained 12% SDS-PAGE gel and Western blot analysis to examine the expression of recombinant TAF protein in Baculovirus.

20 Figure 13A is a schematic illustration of the strategy used in the deletion analysis of *C. albicans* TAF145.

 Figure 13B is a photographic illustration of the Southern blot analysis to determine if the deletion was successful.

Detailed Description of the Invention

25 All patent applications, patents, and literature references cited in this specification are hereby incorporated by reference in their entirety. In case of conflict, the present description, including definitions, will control.

Definitions:

30 "Basal transcription" refers to transcription activity from an RNA polymerase II-directed promoter in the absence of an upstream transcriptional activator.

 "Coactivator activity" refers to the activity that allows an upstream transcription factor such as GAL4 or its derivatives to activate transcription from an

5 RNA polymerase II-directed promoter in an *in vitro* or *in vivo* reconstituted transcription system. Coactivator activity is further defined as an activity that has no effect on basal transcription.

"TATA-box binding protein" or "TBP" is a major component of eukaryotic transcription factors. In fungi and in higher eukaryotes, TBP is isolated as
10 part of a larger protein complex.

"TATA-box binding protein- associated factors" or "TAFs" as used herein refers to polypeptides or complexes of polypeptides required for "coactivator activity" in fungal RNA polymerase II transcription reactions by virtue of their association with TBP.

15 "Functional homology" between TAF polypeptides or complexes of polypeptides indicates that one or more biochemical properties specific to fungal TAFs are shared. Examples of such properties are: the ability to specifically modulate the transcription from RNA polymerase II-directed promoters in the presence of an upstream activator protein, the capacity to specifically bind TBP as a multisubunit
20 complex or as a single subunit under conditions as described herein; and the presence of histone acetyl (HAT) transferase activity.

"TAF subunits" refers to individual polypeptides that comprise the TAF complex activity. Such polypeptides are distinguished from any polypeptides previously known to be TBP binding proteins. Fungal TAF subunits may be
25 recombinant or purified from natural sources, and may include structural or functional TAF homologues as defined above.

A "fungal-specific epitope" of a fungal TAF subunit comprises a three-dimensional structural conformation presented by a folded or assembled TAF polypeptide that is not presented by the homologous mammalian sequence.

30 "Modulating transcription" means altering transcription, and includes increasing or decreasing the rate or level of transcription and changing the responsiveness of transcription to regulatory controls.

An "isolated" polypeptide or nucleic acid is defined as one that is

5 unaccompanied by at least some of the material with which it is associated in its natural state. Generally, an isolated polypeptide constitutes at least about 1%, preferably at least about 10%, and more preferably at least about 50% by weight of the total protein in a given sample. Included in the polypeptide weight are alternative forms such as differentially glycosylated or phosphorylated or otherwise post-
10 translationally modified forms. An "isolated" nucleic acid sequence is present as other than a naturally occurring chromosome or transcript in its natural state and typically is removed from at least some of the proteins with which it is normally associated on a natural chromosome. A partially pure nucleotide sequence constitutes at least about 5%, preferably at least about 30%, and more preferably at least about 90% by weight
15 of total nucleic acid present in a given fraction.

Also encompassed by the invention are nucleic acids that are hybridizable to, or derived from, the TAF-145 sequences described above. In one embodiment, the invention relates to isolated nucleic acids capable of hybridizing with the TAF-145 sequences above or with their complements under the hybridization
20 conditions defined below.

-- Prehybridization treatment of the support (nitrocellulose filter or nylon membrane), to which is bound the nucleic acid capable of hybridizing with that of *C. albicans* TAF-145, at 65°C for 6 hours with a solution having the following composition: 4 x SSC, 10 x Denhardt (1X Denhardt is 1% Ficoll, 1%
25 polyvinylpyrrolidone, 1% BSA (bovine serum albumin); 1 x SSC consists of 0.15M of NaCl and 0.015M of sodium citrate, pH 7);

-- Replacement of the pre-hybridization solution in contact with the support by a buffer solution having the following composition: 4 x SSC, 1 x Denhardt, 25 mM NaPO₄, pH 7, 2 mM EDTA, 0.5% SDS, 100 µg/ml of sonicated
30 salmon sperm DNA containing a nucleic acid derived from the sequence of the TAF-145 as probe, in particular a radioactive probe, and previously denatured by a treatment at 100°C for 3 minutes;

-- Incubation for 12 hours at 65°C;

5 -- Successive washings with the following solutions: (i) four washings with 2 x SSC, 1 x Denhardt, 0.5% SDS for 45 minutes at 65°C; (ii) two washings with 0.2 x SSC, 0.1 x SSC for 45 minutes at 65°C; and (iii) 0.1 x SSC, 0.1% SDS for 45 minutes at 65°C.

10 The invention also encompasses any nucleic acid exhibiting the property of hybridizing specifically with the above-described *C. albicans* TAF-145 under the conditions described above, but at 40°C, including successive washings in 2X SSC at 45°C for 15 minutes.

15 It will be understood that the conditions of hybridization defined above constitute preferred conditions for the hybridization, but are in no way limiting and may be modified without in any way affecting the properties of recognition and hybridization of the probes and nucleic acids mentioned above.

20 The salt conditions and temperature during the hybridization and the washing of the membranes can be modified in the sense of a greater or lesser stringency without the detection of the hybridization being affected. For example, it is possible to add formamide in order to lower the temperature during hybridization.

25 Nucleic acids that hybridize to the TAF-145 sequences of the invention may be of any length. In one embodiment, such polynucleotides are at least 25, preferably at least 100 and most preferably at least 200 nucleotides long. In another embodiment, the polynucleotide that hybridizes to the polynucleotide of the invention is of the same length as the polynucleotide of the invention.

TAF-145-Encoding Nucleic Acids and Polypeptides

30 The present invention encompasses nucleic acid sequences that encode TAF-145 from *C. albicans* and related *Candida* species. Methods for determining the relevant nucleic acid sequences are described in Example 1 below, and the deduced amino acid sequences of a TAF-145 gene, i.e. a gene encoding the 145 kDa TAF polypeptide (TAF-145) isolated from *C. albicans*, is shown in Figure 3. The present invention encompasses DNA and RNA sequences, and sense and antisense sequences.

5 TAF-encoding sequences according to the present invention may be modified by transitions, transversions, deletions, insertions, or other modifications such as alternative splicing. The invention also encompasses genomic TAF-145 sequences and TAF-145 gene flanking sequences, including TAF-145 regulatory sequences. Nucleic acid sequences encoding TAF-145 polypeptides may also be associated with
10 heterologous sequences, including promoters, enhancers, response elements, signal sequences, polyadenylation sequences, introns, 5'- and 3'- noncoding regions, and the like. Other useful heterologous sequences are known to those skilled in the art. Furthermore, the nucleic acids can be modified to alter stability, solubility, binding affinity and specificity. For example, TAF-145 encoding sequences can be selectively
15 methylated. The nucleic acid sequences of the present invention may also be modified with a label capable of providing a detectable signal, either directly or indirectly. Exemplary labels include radioisotopes, fluorescent molecules, biotin, and the like.

In general, nucleic acid manipulations according to the present
20 invention use methods that are well known in the art, as disclosed in e.g. *Molecular Cloning, A Laboratory Manual* (2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor), or *Current Protocols in Molecular Biology* (Eds. Ausubel, Brent, Kingston, More, Feidman, Smith and Stuhl, Greene Publ. Assoc., Wiley-Interscience, NY, NY, 1992).

25 The *C. albicans* TAF-145 of the present invention has not been disclosed or suggested in the prior art. Although there is some sequence homology between the *C. albicans* (SEQ ID NO:2) and *S. cerevisiae* (SEQ ID NO: 3) TAF-145 molecules in the central region of the protein (48%), there is a much lower sequence identity in the aminoterminal and carboxyterminal domains (19% and 29%,
30 respectively). In addition, there is a 74-amino acid sequence (residues 772 to 845) (SEQ ID NO:11) in *C. albicans* TAF-145, located between the central and C-terminal domains, which is not present in *S. cerevisiae*. Moreover, as shown below, *C. albicans* TAF-145 protein does not complement a *S. cerevisiae* TAF-145 temperature

5 sensitive mutant protein when cells are grown at the restrictive temperature. Thus, the two molecules differ unpredictably with respect to both structure and function.

The invention also provides vectors comprising nucleic acids encoding *C. albicans* TAF-145 and analogs thereof. A large number of vectors, including plasmid and fungal vectors, have been described for expression in a variety of eukaryotic and prokaryotic hosts. Advantageously, vectors may also include a promoter and/or any other transcriptional regulatory sequence operably linked to the TAF-145 encoding portion. The encoded TAF-145 may be expressed by using any suitable vectors and host cells, using methods disclosed or cited herein or otherwise known to those skilled in the relevant art. The particular choice of vector/host is not critical to the invention.

Vectors will often include one or more replication systems for cloning or expression, one or more markers for selection in the host, e.g. antibiotic resistance, and one or more expression cassettes. The inserted TAF-145 coding sequences may be synthesized, isolated from natural sources, prepared as hybrids, etc. Ligation of the coding sequences to the transcriptional regulatory sequences may be achieved by known methods. Suitable host cells may be transformed/transfected/infected by any suitable method including electroporation, CaCl_2 mediated DNA uptake, fungal infection, microinjection, microprojectile, or other established methods.

Appropriate host cells include bacteria, archebacteria, fungi, especially yeast, and plant and animal cells, especially mammalian cells. Of particular interest are *E. coli*, *B. Subtilis*, *Saccharomyces cerevisiae*, *Pichia pastoris*, *Candida albicans*, other *Candida* species such as *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. glabrata*. *Aspergillus* species, SF9 cells, C129 cells, 293 cells, *Neurospora*, CHO cells, COS cells, HeLa cells, and immortalized mammalian myeloid and lymphoid cell lines. Preferred replication systems include M13, ColE1, SV40, baculovirus, lambda, adenovirus, and the like. A large number of transcription initiation and termination regulatory regions have been isolated and shown to be effective in the transcription and translation of heterologous proteins in the various hosts. Examples

5 of these regions, methods of isolation, manner of manipulation, etc. are known in the art. Under appropriate expression conditions, host cells can be used as a source of recombinantly produced TAF-145.

Nucleic acids encoding TAF-145 polypeptides may also be introduced into cells by recombination events. For example, such a sequence can be introduced
10 into a cell, and thereby effect homologous recombination at the site of an endogenous gene encoding TAF-145, an analog or pseudogene thereof, or a sequence with substantial identity to a TAF-145-encoding gene. Other recombination-based methods such as nonhomologous recombinations, deletion of endogenous gene by homologous recombination, especially in pluripotent cells, may also be used.

15 The present invention encompasses TAF complexes and subunits purified from wild-type and genetically altered strains of *C. albicans* or recombinantly produced in a non-native context. Yeast TAF complexes comprise about nine polypeptides, or closely related families of polypeptides. The complexes, and polypeptide components thereof, may be isolated by virtue of their affinity for fungal
20 or human TBP, by the use of chromatographic procedures that take advantage of physico-chemical characteristics of the complexes or of individual subunits, or by binding to TAF-specific antibodies. The isolated complexes may contain all, or only a subset, of the total known complement of TAF subunits. TAF multisubunit complexes may also be reconstituted and purified from translation products of subunit
25 genes, or from recombinantly produced TAF subunits. It is also contemplated that additional TAF subunit polypeptides will be identified using methods disclosed herein, and will be used in practicing the present invention.

In one embodiment, a baculovirus expression system permits the recombinant TAF-145 to be modified, processed and transported within a eukaryotic
30 system. In another embodiment, assembly of the TAF complex, or binding of preassembled TAF complexes to TBP, is performed in a reconstituted cell-free system using partially purified or substantially purified components. For example, TAF complexes, or components thereof, may be adsorbed to the surface of a microtiter

5 plate, and incubated with radiolabelled TBP protein. Functional binding of TBP to TAF complexes or components will result in the association of detectable radioactivity with the plate.

C. albicans TAF-145 according to the invention may be isolated from wild-type or mutant fungal cells, or from heterologous organisms or cells (including, but not limited to, bacteria, fungi, insect, plant, and mammalian cells) including fungal cells into which a fungal-derived protein-coding sequence has been introduced and expressed. Furthermore, the TAF-145 sequence may be part of recombinant fusion proteins. Alternatively, TAF-145 polypeptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis.

Methods for polypeptide purification are well-known in the art, including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. For some purposes, it is preferable to produce TAF-145 in a recombinant system in which the fungal protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence. The polypeptide can then be purified from a crude lysate of the host cell by chromatography on an appropriate solid-phase matrix. Alternatively, antibodies produced against TAF-145 or against peptides derived therefrom can be used as purification reagents. Other purification methods are possible.

The present invention also encompasses derivatives and homologues of *C. albicans* TAF-145. For some purposes, nucleic acid sequences encoding the peptides may be altered by substitutions, additions, or deletions that provide for functionally equivalent molecules, i.e., function-conservative variants. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of similar properties, such as, for example, positively charged amino acids (arginine, lysine, and histidine); negatively charged amino acids (aspartate and

5 glutamate); polar neutral amino acids; and non-polar amino acids.

TAF-145 polypeptides can be modified by methods known in the art. For example, TAF-145 may be phosphorylated or dephosphorylated, glycosylated or deglycosylated, and the like. Especially useful are modifications that alter TAF-145 solubility, membrane transportability, stability, and binding specificity and affinity.

10 Some examples include fatty acid-acylation, proteolysis, and mutations in TBP interaction domains that stabilize binding.

TAF-145 of the present invention may also be modified with a label capable of providing a detectable signal, for example, at a heart muscle kinase labeling site, either directly or indirectly. Exemplary labels include radioisotopes,
15 fluorescent compounds, etc. Such labeled TAFs thereof find use, for example, as probes in expression screening assays for proteins that interact with TAF, or in assays for TAF binding to TBP.

Identification of Functionally Important TAF Domains and Binding Partners

20 The polypeptides, protein complexes, and nucleic acids sequences of the present invention find use in the discovery, design, and development of pharmaceutically useful antifungal agents. The following embodiments of the present invention are directed towards elucidating epitopes and interactions of TAF-145 that can be selectively interfered with in a therapeutically beneficial manner.

25 In one embodiment, the known sequence of TAF-145 is used to design synthetic peptides comprising portions of the sequence. These peptides range from about 15 to about 50 amino acids in length. Peptides under 60 amino acids in length may be synthesized routinely using commercially available automated synthesizers. The peptides are then added to a cell-free assembly reaction containing, e.g.,
30 immobilized TAF complexes and soluble radiolabelled TBP. Determining which synthetic peptides inhibit some interaction of TAF-145 e.g. with other TAF subunits or with other factors, using routine experimentation, identifies different functional domains or epitopes of TAF-145. For example, a peptide fragment derived from

5 TAF-145 that is found using the above-described method to inhibit the binding of
TBP to TAF-145 or to a TAF complex is likely to represent a region of TAF-145 that
interacts directly with TBP. In a similar manner, associational domains of different
TAF subunits that are involved in interactions among subunits, or between TAF
subunits or complexes and other transcriptional components, may be systematically
10 identified. These peptides may themselves constitute useful therapeutic reagents, or
may serve as the basis for design and formulation of pharmacologically active
compositions.

In another embodiment, important functional domains of TAFs are
identified using classical and reverse genetic methods that are well-known in the art.
15 For example, a nested set of deletion mutants can be prepared from the TAF-145
sequence. In this embodiment, progressively longer amino-terminal and carboxy-
terminal deletions can be engineered in the TAF-145 sequence. The resulting set of
mutant sequences can be individually expressed in a fungal strain under conditions in
which the wild-type version of the TAF is not expressed (see, e.g., Example 1 below,
20 in which *S. cerevisiae* was used as a host). By monitoring the function of each
mutant, it is possible to identify different regions of the TAF-145 polypeptide that are
critical for function i.e. functional domains or epitopes. Based on such studies, using
methods that are well-known in the art, it is possible to selectively introduce defined
mutations into different regions of the polypeptide and characterize the variant
25 protein's activity using a similar functional analysis.

An important aspect of the present invention is the selection of
functionally important domains or epitopes of *Candida* TAF-145 subunits that are
structurally and/or functionally distinct from their mammalian homologues. Such
domains are particularly useful as targets for antifungal drugs. In the case of TAF-
30 145, the *Candida* version differs in several important respects from its human
homologue, TAF-250. *Candida* TAF-145 is approximately half the size of human
TAF-250, and the homologous regions display an amino acid similarity and identity
of only 58% and 33%, respectively. *Candida* TAF-145 lacks the carboxy terminal

- 5 half of its human counterpart that contains the proposed "Bromo domains" and a region rich in acidic amino acid residues.

Identification of important structural and functional domains of TAFs according to the present invention enables the design and production of useful TAF-derived nucleic acid and peptide-based compounds. For example, fusion proteins may
10 be produced between an important TAF domain and e.g., an enzymatically active fragment of a DNA endonuclease. The resulting fusion protein, which can be produced in a fungal cell following introduction into the cell of the hybrid DNA operably linked to an expression vector, finds use in modulating TAF-dependent gene transcription. Other useful TAF fusion partners include sequences useful for
15 immobilization. For example, sequences derived from glutathione-S-transferase (GST) provide a binding site for immobilized glutathione, and sequences that form an epitope recognized by an available monoclonal antibody (e.g., 12CA5 monoclonal antibody) provide a binding site for the immobilized antibody.

In another example, particular serine, threonine, or tyrosine residues
20 in a TAF sequence may be identified as functionally important sites for phosphorylation of TAF. See e.g., methods disclosed in Roberts et al. (1991) *Science* **253**, 1022-1026, and in Wegner et al. (1992) *Science* **256**, 370-373. Phosphorylation of TAF subunits may be involved in modulating the transcription activation activity of Polymerase II transcribed genes. Identification of these residues will enable, first, the
25 radiolabelling of TAF subunits with γ -³²P-ATP. Furthermore, if phosphorylation of a particular residue is necessary for transcriptional activity, phosphorylation inhibitors may be designed to block activity.

The nucleic acids encoding TAF-145 may also be used to identify other nuclear factors that interact with TAF-145. In this embodiment, a yeast cDNA
30 library containing fusion genes of cDNA joined with DNA encoding the activation domain of a transcription factor (e.g., Gal4) is co-transfected with fusion genes encoding a portion of TAF and the DNA binding domain of a transcription factor. Clones encoding TAF binding proteins are able to complement the transcription factor

- 5 and are identified through transcription of a reporter gene. See, e.g., Fields et al. (1989) *Nature* **340**: 245-246, and Chien et al., (1991) *Proc. Natl. Acad. Sci. USA* **88**:9578-9582. It is contemplated that these additional binding partners for TAF will provide additional targets for antifungal drug therapy.

10 **Anti-TAF Antibodies**

- The present invention encompasses antibodies that are specific for TAF-145 complexes or subunits identified as described above. The antibodies may be polyclonal or monoclonal, and may distinguish TAFs from other nuclear proteins, discriminate TAFs from those derived from different species, identify associational or
- 15 other functional domains, and the like. Such antibodies are conveniently made using the methods and compositions disclosed in Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, other references cited herein, as well as immunological and hybridoma technologies known to those in the art. Where natural or synthetic TAF-derived peptides are used to induce an TAF-
- 20 specific immune response, the peptides may be conveniently coupled to an suitable carrier such as KLH and administered in a suitable adjuvant such as Freund's. Preferably, selected peptides are coupled to a lysine core carrier substantially according to the methods of Tam (1988) *Proc. Natl. Acad. Sci. USA* **85**:5409-5413. The resulting antibodies may be modified to a monovalent form e.g. Fab, FAB', or
- 25 FV. Anti-idiotypic antibodies, especially internal imaging anti-idiotypic antibodies, may also be prepared using known methods.

- In one embodiment, purified *C. albicans* TAF-145 is used to immunize mice, after which their spleens are removed, and splenocytes used to form cell hybrids with myeloma cells and obtain clones of antibody-secreting cells according to
- 30 conventional techniques. The resulting monoclonal antibodies are screened using *in vitro* assays such as those described above for the following activities: binding to TAF-145, inhibition of TAF-145 incorporation into multimeric TAF complexes, and inhibition of TAF-145 - TBP interaction.

5 In another embodiment, the entire TAF complex is used as an immunogen as above, and the resulting monoclonal antibodies are screened for their activity in inhibiting the *in vitro* assembly of any component of the TAF complex.

Anti-TAF antibodies may be used to identify and quantify TAF components, using immunoassays such as ELISA, EMIT, CEDIA, SLIFA, and the
10 like. Anti-TAF antibodies may also be used to block the transcriptional function of, e.g., TAF-145 by inhibiting formation of complexes between TAF subunits or between assembled TAF complexes and other transcription components, or by immunodepleting cell extracts or transcription reactions of TAF components. In addition, these antibodies can be used to identify, isolate, and purify TAFs from
15 different sources, and to perform subcellular and histochemical localization studies.

In one embodiment, polyclonal antibodies against the HAT domain of *C. albicans* TAF145 (aa 339-766) were generated by injection of purified recombinant protein into rabbits (Robert Sargeant, Ramona CA). The sera from these rabbits were then screened by ELISA (Figure 11) for their ability to recognize the recombinant
20 CaTAF145 HAT domain (Example 5)

High-Throughput Drug Screening

The present invention encompasses the identification of agents useful in modulating fungal gene transcription, particularly the transcription of genes by
25 RNA Polymerase II in a TAF-dependent manner. In a preferred embodiment, a high-throughput screening protocol is used to survey a large number of test compounds for their ability to interfere with TAF-dependent processes.

Test inhibitory compounds are screened from large libraries of synthetic or natural compounds. Numerous means are currently used for random and
30 directed synthesis of saccharide, peptide, and nucleic acid based compounds. Synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Brandon Associates (Merrimack, NH), and Microsource (New Milford, CT). A rare chemical library is

5 available from Aldrich (Milwaukee, WI). Alternatively, libraries of natural
compounds in the form of bacterial, fungal, plant and animal extracts are available
from e.g. Pan Laboratories (Bothell, WA) or MycoSearch (NC), or are readily
produceable. Additionally, natural and synthetically produced libraries and
10 compounds are readily modified through conventional chemical, physical, and
biochemical means.

Useful inhibitory agents are identified using any suitable assay that
employs TAF-145 or TAF-encoding nucleic acids. As examples, protein binding
assays, nucleic acid binding assays and gel shift assays are useful approaches.

15 In one set of embodiments, TAF complexes or TAF-145 as provided
by the present invention are to be used in *in vitro* binding assays with either TBP
alone or with a combination or subcombination of TBP and general transcription
factors (GTFs).

For example, TAF complexes or TAF-145 may be immobilized on
microtiter dishes using methods that are standard in the art. The plates are then
20 exposed to radiolabelled TBP e.g. [³²P]-TBP in the absence or presence of candidate
compounds. Conversely, TBP may be immobilized, and incubated with radiolabelled
TAF-145 or TAF complexes in the absence or presence of candidate compounds.
Oligonucleotides comprising TBP target sequences may be used in conjunction with
TBP and TAF. Postive "hit" compounds are those that inhibit TAF-TBP interaction.
25 In this case, incubation, washing, and radioactivity detection steps can be automated,
allowing the screening of a large number of compounds, preferably at least about
1000 compounds per week.

In another embodiment, test compounds are screened to identify those
that inhibit the histone acetyltransferase (HAT) activity of TAF-145 (see, e.g.,
30 Examples 2 and 3 below). Positive "hit" compounds are those that cause at least
about 25%, preferably at least about 50%, and most preferably at least about 75%,
inhibition of incorporation of [3H]-acetate into histones using the reaction conditions
described in Example 2 below.

5 In another embodiment, test compounds are screened to identify those that bind TAF-145, using the high-throughput screening methods described in U.S. Patent Nos. 5,585,277 and 5,679,582, in U.S.S.N. 08/547,889, and in PCT published application PCT/US96/19698. These methods may be used for identifying a ligand that binds the TAF 145 protein. According to these methods, a ligand, or a plurality of ligands for TAF 145 target protein is identified by its ability to influence the extent of folding or the rate of folding or unfolding of the target protein. Experimental conditions are chosen so that the target protein is subjected to unfolding, whether reversible or irreversible. If the test ligand binds to the target protein under these conditions, the relative amount of folded:unfolded target protein or the rate of folding or unfolding of the target protein in the presence of the test ligand will be different, i.e. higher or lower, than that observed in the absence of the test ligand. Thus, the method encompasses incubating TAF 145 in the presence and absence of a test ligand or ligands, under conditions in which (in the absence of ligand) the TAF 145 protein would partially or totally unfold. This is followed by analysis of the absolute or relative amounts of folded vs. unfolded target protein or of the rate of folding or unfolding of the target protein.

An important feature of this method is that it will detect any compound that binds to any sequence or domain of the TAF 145, and not only to sequences or domains that are intimately involved in a biological activity or function. The binding sequence, region, or domain may be present on the surface of the TAF 145 when it is in its folded state, or may be buried in the interior of the protein. Some binding sites may only become accessible to ligand binding when the protein is partially or totally unfolded.

Briefly, to carry out this method, the test ligand or ligands are combined with the TAF 145, and the mixture is maintained under appropriate conditions and for a sufficient time to allow binding of the test ligand. Experimental conditions are determined empirically. When testing test ligands, incubation conditions are chosen so that most ligand:TAF 145 protein interactions would be

5 expected to proceed to completion. The test ligand is present in molar excess relative to the TAF 145. The target protein can be in a soluble form, or, alternatively, can be bound to a solid phase matrix. The matrix may comprise without limitation beads, membrane filters, plastic surfaces, or other suitable solid supports.

In a preferred embodiment, binding of test ligand or ligands to TAF
10 145 is detected through the use of proteolysis. This assay is based on the increased susceptibility of unfolded, denatured polypeptides to protease digestion relative to that of folded proteins. In this case, the test ligand-TAF 145 protein combination, and a control combination lacking the test ligand, are treated with one or more proteases that act preferentially upon unfolded target protein. After an appropriate period of
15 incubation, the level of intact i.e. unproteolysed target protein is assessed using one of the methods described below e.g. gel electrophoresis and/or immunoassay.

There are two possible outcomes that indicate that the test ligand has bound the target protein. Either 1) a significantly higher, or 2) a significantly lower, absolute amount of intact or degraded protein may be observed in the presence of
20 ligand than in its absence.

Proteases useful in practicing the present invention include without limitation trypsin, chymotrypsin, V8 protease, elastase, carboxypeptidase, proteinase K, thermolysin, papain and subtilisin (all of which can be obtained from Sigma Chemical Co., St. Louis, MO). The most important criterion in selecting a protease or
25 proteases for use in practicing the present invention is that the protease(s) must be capable of digesting the TAF 145 protein under the chosen incubation conditions, and that this activity be preferentially directed towards the unfolded form of the protein. To avoid "false positive" results caused by test ligands that directly inhibit the protease, more than one protease, particularly proteases with different enzymatic
30 mechanisms of action, can be used simultaneously or in parallel assays. In addition, co-factors that are required for the activity of the protease(s) are provided in excess, to avoid false positive results due to test ligands that may sequester these factors.

In a typical embodiment of this method, purified TAF 145 protein is

- 5 first taken up to a final concentration of 1-100 µg/ml in a buffer containing 50 mM Tris-HCl, pH 7.5, 10% DMSO, 50 mM NaCl, 10% glycerol, and 1.0 mM DTT. Proteases, such as, for example, proteinase K or thermolysin (proteases with distinct mechanisms of action), are then added individually to a final concentration of 0.2-10.0 µg/ml. Parallel incubations are performed for different time periods ranging from 5
- 10 minutes to one hour, preferably 30 minutes, at 4°C, 15°C, 25°C, and 35°C. Reactions are terminated by addition of an appropriate protease inhibitor, such as, for example, phenylmethylsulfonyl chloride (PMSF) to a final concentration of 1mM (for serine proteases), ethylenediaminetetraacetic acid (EDTA) to a final concentration of 20 mM (for metalloproteases), or iodoacetamide (for cysteine proteases). The amount
- 15 of intact protein remaining in the reaction mixture at the end of the incubation period may then be assessed by any method, including without limitation polyacrylamide gel electrophoresis, ELISA, or binding to nitrocellulose filters. It will be understood that additional experiments employing a narrower range of temperatures can be performed to establish appropriate conditions. This protocol allows the selection of appropriate
- 20 conditions (e.g., protease concentration and digestion temperature) that result in digestion of approximately 70% of the target protein within a 30 minute incubation period, indicating that a significant degree of unfolding has occurred.

In another embodiment, the relative amount of folded and unfolded TAF 145 protein in the presence and absence of test ligand is assessed by measuring

25 the relative amount of the protein that binds to an appropriate surface. This method takes advantage of the increased propensity of unfolded proteins to adhere to surfaces, which is due to the increased surface area, and decrease in masking of hydrophobic residues, that results from unfolding. If a test ligand binds the TAF 145 (i.e., is a ligand), it may stabilize the folded form of the target protein and decrease its binding

30 to a solid surface. Alternatively, a ligand may stabilize the unfolded form of the protein and increase its binding to a solid surface.

Surfaces suitable for this purpose include without limitation microtiter plates constructed from a variety of treated or untreated plastics, plates treated for

5 tissue culture or for high protein binding, nitrocellulose filters and PVDF filters.

In another embodiment, the extent to which folded and unfolded target protein are present in the test combination is assessed through the use of antibodies specific for either the unfolded state or the folded state of the protein i.e. denatured-specific ("DS"), or native-specific ("NS") antibodies, respectively. (Breyer, 1989, *J. Biol. Chem.*, **264** (5):13348-13354). Polyclonal or monoclonal antibodies are prepared as described above. The resulting antibodies are screened for preferential binding to the TAF 145 protein in its denatured state. These antibodies are used to screen for inhibitors of these interactions.

In another embodiment, molecular chaperones are used to assess the relative levels of folded and unfolded protein in a test combination. Chaperones encompass known proteins that bind unfolded proteins as part of their normal physiological function. In this embodiment, a test combination containing the test ligand and the TAF 145 is exposed to a solid support e.g. microtiter plate or other suitable surface coated with a molecular chaperone, under conditions appropriate for binding the TAF 145 with its ligand and binding of the molecular chaperone to unfolded target protein. The unfolded target protein in the solution will have a greater tendency to bind to the molecular chaperone-covered surface relative to the ligand-stabilized folded target protein. Thus, the ability of the test ligand to bind target protein can be determined by determining the amount of target protein remaining unbound, or the amount bound to the chaperone-coated surface. Alternatively, a competition assay for binding to molecular chaperones can be utilized.

Once conditions are established for high-throughput screening as described above, the protocol is repeated simultaneously with a large number of test ligands at concentrations ranging from 20 to 200 μ M. Observation of at least a two-fold increase or decrease in the extent of digestion of the target protein signifies a "hit" compound, i.e., a ligand that binds the target protein. Preferred conditions are those in which between 0.1% and 1% of test ligands are identified as "hit" compounds using this procedure.

5 In yet another embodiment, the test and control combinations described above can be contacted with a conformation-sensitive fluorescence probe, i.e., a probe that binds preferentially to the folded, unfolded, or molten globule state of the TAF 145 or whose fluorescence properties are in any way affected by the folding status of the TAF 145 protein. Once a particular test compound has been identified as
10 described above, its activity is then confirmed by adding it to an *in vitro* transcription reaction, and measuring its effect on TAF-mediated activated transcription.

It is also contemplated that a useful agent may interfere with the function of TAF-145 but not inhibit TAF-TBP complex assembly. To screen for such compounds, other functional assays are used, such as, e.g., *in vitro* transcription
15 reactions.

Finally, a test compound identified as described above is tested for two properties: its ability to inhibit fungal growth and its lack of effect on mammalian transcription. Fungal growth is measured by any method well-known in the art e.g. optical density of a liquid culture, or colony formation on agar. The lack of effect of a
20 test compound on mammalian TAF-TBP interaction is tested by replacing yeast components with an analogous human *in vitro* transcription system as in, e.g., Manley et al. (1980) *Proc. Natl. Acad. Sci. USA* 77:3855-3859.

It will be understood that a compound that interferes with any aspect of TAF assembly or function is a likely candidate for an antifungal drug. Thus, in a
25 manner similar to that described above for TBP-TAF-145 binding paradigm, binding assays can be routinely devised that measure the interaction of two or more TAF subunits with each other, or the interaction of one or more TAF subunits with other necessary transcription factors.

According to the present invention, useful agents may be found within
30 numerous chemical classes, though typically they are organic compounds, and preferably small organic compounds. Small organic compounds have a molecular weight of more than 50 yet less than about 2,500 daltons, preferably less than about 750, more preferably less than about 250 daltons. Exemplary classes include

5 peptides, saccharides, steroids, and the like. The compounds may be modified to enhance efficacy, stability, pharmaceutical compatibility, and the like. Structural identification of an agent may be used to identify, generate, or screen additional agents. For example, where peptide agents are identified, they may be modified in a variety of ways to enhance their stability, such as using an unnatural amino acid, such
10 as a D-amino acid, particularly D-alanine, by functionalizing the amino or carboxyl terminus, e.g., for the amino group, acylation or alkylation, and for the carboxyl group, esterification or amidification, or the like. Other methods of stabilization may include encapsulation, for example, in liposomes, etc.

15 **Therapeutic Applications**

For therapeutic uses such as the treatment of fungal infections in mammals, the compositions and agents disclosed herein may be administered by any convenient way, such as, e.g., parenterally, conveniently in a physiologically acceptable carrier, such as, e.g., phosphate buffered saline, saline, deionized water, or
20 the like. Typically, the compositions are added to a retained physiological fluid such as blood or synovial fluid. Alternatively, the compositions may comprise creams, ointments, lotions, or sprays for topical use. Generally, the amount administered will be empirically determined, typically in the range of about 10 to 1000 pg/kg of the recipient. For peptide agents, the concentration of will generally be in the range of
25 about 100 to 500 µg/ml in the dose administered. Other additives may be included, such as stabilizers, bactericides, etc. These additives will be present in conventional amounts.

Example 1: Cloning and Characterization of *Candida albicans* TAF-145

30 **A. Degenerate oligonucleotide PCR**

A protein sequence alignment of the *Saccharomyces cerevisiae* (SEQ ID NO: 3) and TAF-145 counterparts from *Schizosaccharomyces pombe* (SEQ ID NO: 4), *Drosophila* (SEQ ID NO:5) , and human (SEQ ID NO: 6) is shown in Figures 1A-1C.

Three highly conserved regions are boxed. Based on the sequence of these highly conserved regions, degenerate oligonucleotides (see Table 1 below) were designed and used to amplify fragments of DNA from *Candida albicans* strain SC5314 genomic DNA by polymerase chain reaction (PCR) (Figs. 2A, 2B and SEQ ID NO:12 - SEQ ID NO:15).

PCR reactions were performed with Taq DNA polymerase (Promega) in 25 µl of buffer (provided by manufacturer) supplemented with 2 mM MgCl₂ and 200 µM dNTP for a total of 30 cycles. Primers (shown in Table 1 below) were used at a concentration of 1.0 µM with 100 ng of DNA and cycling was at 94°C (45 sec), 37°C (1.0 minute), and 72°C (1 minute) for four cycles followed by 94°C (45 sec), 40°C (1.0 minute), and 72°C (1 minute) for 26 cycles.

TABLE 1: Primers

Oligonucleotide	Sequence	Direction	Corresponding <i>S. cerevisiae</i> amino acids	SEQ ID NO:
TAF145p1F	5'-CC(A/T)GG(A/T)CC(A/T)AA(C/T) TCIA(A/G)(A/G)-3'	Forward	668-673	12
TAF145p2F	5'-GA(C/T)CC(A/T)AC(T/C/A)GG (A/T)TGTGG(A/T)GAAGG-3'	Forward	817-824	13
TAF145p3R	5'-CCTTTC(A/T)CCACA(A/T)CCAGT (A/T)GG(A/G)TC-3'	Reverse	817-824	14
TAF145p4R	5'-TT(A/G)TT(A/C/T)CA(C/T)CTIA (G/A)TG(T/A)CC-3'	Reverse	1045-1051	15

A major 700 bp fragment of DNA was amplified by use of the TAF145p1F and TAF145p3R primers. The amplified DNA was subcloned into the SrfI site of pCR-Script Amp SK(+) plasmid (Stratagene) and subjected to DNA sequencing (see below).

B. Cloning and sequence analysis of *C. albicans* TAF145

[³²P]-labeled DNA was generated by random hexamer priming (Boehringer Mannheim) of the cloned 700 bp *C. albicans* TAF-145 PCR product. The radiolabeled DNA was used to screen a *C. albicans* genomic DNA cosmid library by colony hybridization (Sambrook et al., 1989). The *C. albicans* genomic DNA

5 cosmid library was constructed using conventional procedures: the genomic DNA used to construct the library was purified from *C. albicans* strain SC5314 (Fonzi and Irwin, 1993) as described by Philippsen et al. (1991).

A 6.3 kb fragment of DNA containing the full length *C. albicans* TAF-145 (CaTAF145) gene was subcloned from a cosmid clone into pCT538, creating
10 pFL207 (see Table 2 below). All DNA manipulations were performed according to Sambrook et al. (1989). Figures 3A-3G display the entire nucleotide sequence of the *C. albicans* TAF145 gene (SEQ ID NO:1) and the predicted amino acid sequence (SEQ ID NO: 2) . *C. albicans* translates the standard leucine CUG codon as serine (Santos et al., 1996). Amino acids 980 and 1085 are shown as serines to reflect this
15 divergence from the standard genetic code.

Figures 4A-4D compare the amino amino acid sequences of *C. albicans* (SEQ ID NO:2) and *S. cerevisiae* (SEQ ID NO: 3) TAF145. While there is some sequence identity (48%) between *C. albicans* and *S. cerevisiae* in the middle portion of the protein, there is much lower sequence identity in the N-terminus and C-terminus portions (19% and 29%, respectively). The middle portion of the protein contains a putative histone acetyltransferase domain (Mizzen et al., 1996). An
20 additional difference between these two protein sequences is a 74-amino acid sequence (residues 772-845) in *C. albicans* TAF-145 (SEQ ID NO:11) , located between the central and C-terminus domains, which is not present in *S. cerevisiae*
25 TAF-145. Figures 5A-5C show a protein sequence alignment of the TAF-145 proteins from three yeast species: *C. albicans* (SEQ ID NO: 2) , *S. cerevisiae* (SEQ ID NO: 3) , and *S. pombe* (SEQ ID NO: 4) , and Figures 6A-6C display an alignment of the TAF-145-related proteins from *C. albicans* (SEQ ID NO:2) , *S. cerevisiae* (SEQ ID NO: 3) , *S. pombe* (SEQ ID NO: 4) , *Drosophila* (SEQ ID NO: 5) , and human
30 (SEQ ID NO: 6) . All sequence alignments were done with the Lasergene software package from DNASTAR Inc. The alignments illustrate the divergence of the aminoterminal and carboxyterminal domains of these proteins relative to the central domain.

5 C. Functional analysis of *Candida albicans* TAF-145

Two experiments were performed to determine if a genomic clone of *C. albicans* TAF-145 (CaTAF145) could substitute functionally for *S. cerevisiae* TAF-145 (ScTAF145). Plasmids and yeast strains used in these experiments are described in Tables 2 and 3, respectively. Yeast media, plasmids, and strains were prepared as described (Guthrie and Fink, 1991). Yeast transformations were done using a lithium acetate procedure (Schiestl and Gietz, 1989). Plasmid shuffle techniques were performed as described (Boeke et al., 1987) using 5-fluoro-orotic acid (5-FOA) as a selective agent against URA3 plasmids. All DNA manipulations used to generate the necessary plasmids were performed according to Sambrook et al. (1989).

TABLE 2

Plasmid	Description
pgTAF145	URA3 CEN ScTAF145
pTAF145 ^{ts-2}	HIS3 CEN ScTAF145 ^{ts-2}
pCT538	LEU2 2 μ
pFL112	LEU2 2 μ ScTAF145
pFL207	LEU2 2 μ CaTAF145
pCT3	URA3 CEN
pFL103	URA3 CEN ScTAF145
pFL115	URA3 CEN CaTAF145

TABLE 3

<i>S. cerevisiae</i> Strain	Genotype
YSW85	Mata lys2-801 ade2-101 leu2- Δ 1 his3- Δ 200 ura3- Δ 99 taf145::LEU2 [pgTAF145 (URA3 CEN ScTAF145)]
YSW101	Mata ade2-101 leu2-3,112 his3-11 ura3-1 trp1-1 taf145::hisG [pgTAF145 (URA3 CEN ScTAF145)]

5	CTY507	Mata lys2-801 ade2-101 leu2-Δ1 his3-Δ200 ura3-Δ99 taf145::LEU2 [pTAF145 ^{ts-2} (HIS3 CEN ScTAF145 ^{ts-2})]
	YFL021	Mata ade2-101 leu2-3,112 his3-11 ura3-1 trp1-1 taf145::hisG [pgTAF145 (URA3 CEN ScTAF145), pFL207 (LEU2 2μ CaTAF145)]
	YFL022	Mata ade2-101 leu2-3,112 his3-11 ura3-1 trp1-1 taf145::hisG [pgTAF145 (URA3 CEN ScTAF145), pFL112 (LEU2 2μ ScTAF145)]
	YFL023	Mata ade2-101 leu2-3,112 his3-11 ura3-1 trp1-1 taf145::hisG [pgTAF145 (URA3 CEN ScTAF145), pCT538 (LEU2 2μ)]
	YFL024	Mata lys2-801 ade2-101 leu2-Δ1 his3-Δ200 ura3-Δ99 taf145::LEU2 [pTAF145 ^{ts-2} (HIS3 CEN ScTAF145 ^{ts-2}), pFL115 (URA3 CEN CaTAF145)]
10	YFL025	Mata lys2-801 ade2-101 leu2-Δ1 his3-Δ200 ura3-Δ99 taf145::LEU2 [pTAF145 ^{ts-2} (HIS3 CEN ScTAF145 ^{ts-2}), pFL103 (URA3 CEN ScTAF145)]
	YFL026	Mata lys2-801 ade2-101 leu2-Δ1 his3-Δ200 ura3-Δ99 taf145::LEU2 [pTAF145 ^{ts-2} (HIS3 CEN ScTAF145 ^{ts-2}), pCT3 (URA3 CEN)]

- One experiment took advantage of a temperature-sensitive (ts) mutant.
- 15 Plasmid shuffle techniques were used to create CTY507 (a *S. cerevisiae* strain with a temperature-sensitive allele of ScTAF145) from YSW85 and pTAF145^{ts-2} (Reese et al., 1994; Walker et al., 1996). CTY507 was then transformed with pCT3 ("empty" vector), pFL103 (ScTAF145), and pFL115 (CaTAF145) to generate *S. cerevisiae* strains YFL026, YFL025, and YFL024, respectively. Agar plates inoculated with
- 20 YFL024, YFL025, and YFL026 were placed at permissive (30°C) and restrictive (37°C) temperatures. At the permissive temperature, all three strains grew equally well (Figure 7A). In contrast, at the restrictive temperature, only cells transformed with wild-type *S. cerevisiae* TAF145 (YFL025) grew. Cells containing a genomic

- 5 clone of *C. albicans* TAF145 (YFL024) were unable to grow at the restrictive temperature.

A second experiment used plasmid shuffle techniques to determine if *C. albicans* TAF-145 could function in place of *S. cerevisiae* TAF-145. *S. cerevisiae* strain YSW101, containing a wild-type version of ScTAF145 on a URA3 plasmid, 10 was transformed with pCT538 (empty vector), pFL112 (ScTAF145), or pFL207 (CaTAF145) to generate *S. cerevisiae* strains YFL023, YFL022, and YFL021, respectively. Cells were placed on agar lacking leucine and selected for *Candida* TAF-145-encoding plasmids either in the absence or presence of 5-FOA (Figure 7B). All three strains grew equally well in the absence of 5-FOA. When the URA3- 15 containing ScTAF145 plasmid was selected against by the presence of 5-FOA, only those cells (YFL022) transformed by ScTAF145 were able to grow. In the absence of a functional *S. cerevisiae* TAF-145, cells containing a genomic clone of *C. albicans* TAF-145 were unable to grow.

Conditional mutant analysis and plasmid shuffle techniques in *S. 20 cerevisiae* show that a genomic clone of *C. albicans* TAF-145 was unable to substitute functionally *in vivo* for *S. cerevisiae* TAF145. In principle, the lack of complementation may be due to a lack of expression of mRNA and/or protein, or to a substitution of serines 980 and 1085 for leucine. A more likely explanation is that one or more of the protein surfaces required for interactions between yeast TAF-145 and 25 its partners may not be conserved between *S. cerevisiae* and *C. albicans*. While the middle portion of the two proteins share significant sequence similarity, the aminoterminal and carboxyterminal domains have diverged considerably (Figures 4A-4D).

D. Expression and purification of recombinant proteins

30 It has recently been reported that the *S. cerevisiae* TAF-145 protein and the human and *Drosophila* counterparts have histone acetyltransferase (HAT) activity associated with them (Mizzen et al., 1996). This activity has been mapped to the middle, most conserved, portion of ScTAF145 (amino acids 354-817) (SEQ ID

NO:8). Figures 8A and 8B display an alignment of the TAF-145 HAT domain from *C. albicans* (SEQ ID NO:7), *S. cerevisiae* (SEQ ID NO:8), *S. pombe* (SEQ ID NO:9), and human (SEQ ID NO:10). The HAT domains derived from the three fungal species share approximately 50% amino acid sequence identity, while there is less than 25% amino acid sequence identity between the fungal and human TAF-145 HAT domains.

The portion of the gene encoding the putative HAT domains from *S. cerevisiae*, *C. albicans*, and human were PCR amplified and subcloned into pET19b (Novagen) to create pFL105, pFL107, and pFL108, respectively. Oligonucleotide sequences (SEQ ID NO:16 - SEQ ID NO:21) are listed in Table 4 below. Plasmids were transformed into *E. coli* BL21(DE3) for protein expression. Upon induction with IPTG, an amino-terminal His tagged protein was expressed.

TABLE 4

Oligo-nucleotide	Organism	Sequence	Direction	Corresponding amino acids and restriction sites created	SEQ ID NO:
pScHAT1	<i>S. cerevisiae</i>	5'-CCGCTCGAGAT GACACCCAACCTAAA GTTC-3'	Forward	amino acid 354 to.... XhoI	16
pScHAT3	<i>S. cerevisiae</i>	5'-CGCGGATCCAGA GATTTTAGCTTAGAA TC-3'	Reverse	amino acid 787 BamHI	17
pCaHAT3	<i>C. albicans</i>	5'-GGAATTC CATATGCTTTTGCTC AACAATCCCTTGGAC -3'	Forward	amino acid 339 to... NdeI	18
pCaHAT4	<i>C. albicans</i>	5'-CGCGGATCCCT GCTCTGCTCACCGAA TAACAC-3'	Reverse	amino acid 766 BamHI	19
pHuHAT 6	Human	5'-GGAATTC CATATGAGCCTGGCA GGCTGGCTTCC TTCT-3'	Forward	amino acid 432 to... NdeI	20
pHuHAT 7	Human	5'-CCGCTCGAGTTC TGGAGCAAAAAGG ATTTCTC-3'	Reverse	amino acid 912 XhoI	21

Restriction enzyme sites are underlined.

5 His-tagged HAT domain fusion proteins were purified on a nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography matrix (Qiagen) according to manufacturers instructions. Cells were lysed and proteins solubilized under denaturing conditions. Proteins were refolded on the Ni-NTA column and eluted with 0.5 M imidazole. Figure 9 shows the protein profile of extracts from
10 uninduced (U) and induced (I) cells following Ni-NTA chromatography. Addition of IPTG induced proteins of the expected size for the *S. cerevisiae* (Sc) and *C. albicans* (Ca) proteins (Figure 9).

Example 2: Histone acetyltransferase assays

15 Two assays for histone acetyltransferase (HAT) activity are described below. The first assay is an activity gel assay that permits direct association of polypeptides with acetyltransferase activity (Brownell and Allis, 1995). Purified HAT proteins are analyzed for HAT activity following electrophoresis in SDS/polyacrylamide gels containing calf thymus histones or bovine serum albumin.
20 Samples are dissolved in SDS/PAGE sample buffer, but not boiled, and then loaded onto standard SDS/polyacrylamide gels which have been modified so that protein substrates are dissolved in the resolving gel at 1 mg/ml prior to polymerization. Following electrophoresis, gels are washed for 1 hour at room temperature in buffer A (50 mM Tris-HCl pH8.0, 20%(v/v) isopropanol, 1 mM DTT, 1 mM PMSF, 0.1 mM
25 EDTA). Gels are then incubated in Buffer A containing 8 M urea for 1 hour and then overnight at 4°C in buffer A containing 0.04% Tween 40 (Sigma). The gels are then washed in buffer B (50 mM Tris-HCl pH8.0, 10%(v/v) glycerol, 1 mM DTT, 1 mM PMSF, 0.1 mM EDTA) prior to incubation with [³H]acetyl-CoA (10 µCi) for 30 minutes at 30°C. Finally, the labeled gels are washed with 5% trichloroacetic acid to
30 remove unbound radiolabel and fluorographed.

The second assay is a standard solution assay (Brownell and Allis, 1995). Enzyme samples are incubated at 30°C in a total volume of 50 µl of buffer B (50 mM Tris-HCl pH8.0, 10%(v/v) glycerol, 1 mM DTT, 1 mM PMSF, 0.1 mM

- 5 EDTA) and 25 mg of calf thymus histones (Sigma). The reactions are initiated by the addition of [³H]acetyl-CoA (100 nCi; 6.1 Ci/mmol; ICN) to a final concentration of 0.5 μM and are terminated after 10 minutes by spotting the entire mixture onto Whatman P81 filters. [³H]-Acetate incorporation is determined by liquid scintillation and nonspecific counts are subtracted. This assay can be adapted for a high throughput
- 10 screen for the identification of compounds that inhibit the activity of fungal TAF145 acetyltransferase activity. A counterscreen with the human HAT domain protein is used to identify those inhibitors specific for the fungal proteins.

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40

Example 3: High-throughput Screening of Anti-TAF compounds

A. TAF binding

Corning ELISA strip wells (8 wells per strip) are coated with avidin (1.0 µg per well) by incubating avidin (200 µl of a 5 µg/ml stock) in coupling buffer (per liter: 1.6g Na₂CO₃, 2.9 g, NaHCO₃, 0.9 g NaN₃) in the well for 12 h at 4°C. The

45

5 buffer is decanted, and nonspecific binding sites on the wells are blocked with 1% skim milk in phosphate-buffered saline (PBS) for 1 h at 37°C. Blocking buffer is discarded, and a yeast Pol II promoter-containing oligonucleotide (1 pmol/well) is added to the wells and incubated for 30 minutes at room temperature. The oligonucleotide is double-stranded and contains a biotin tag on the sense strand.

10 The oligonucleotide-containing solution is then removed, and the wells are washed with 1% milk in PBS. Yeast TBP is mixed with partially purified TAF that had been metabolically labelled with ³⁵S-methionine or purified TAF-145 similarly labelled, all in HEG buffer (0.1 M KCl, 25 mM HEPES pH 7.9, 0.5 mM EDTA, 20% glycerol, 0.01% LDAO, 0.1 M AEBSF, 0.1 M Na metabisulfite, 10 mM
15 β-mercaptoethanol) plus 200 ug/ml bovine serum albumin (BSA).

The protein mixture is then added to the prepared wells and incubated for 30 minutes at room temperature. Samples are then removed, and the wells are washed three times with the PBS/milk solution. Wells are separated and put into scintillation vials, scintillation cocktail is added, and samples are counted in a liquid
20 scintillation counter.

Binding of yeast TAF to the wells is found to be dependent on the presence of TBP, bound in turn to the Pol II promoter-containing oligonucleotide. Small molecules, whether purified or present in natural or synthetic mixtures, are introduced into the assay at concentrations ranging from about 20 to about 200 μM,
25 and appropriate solvent controls are also performed. Compounds that inhibit binding of TAF by more than about 30% are identified, and the inhibitory activity purified if not already available in pure form.

Compounds identified as described above are then tested for their ability to inhibit TBP-dependent transcription in a mammalian cell-free system.
30 Alternatively, human TBP is used in place of yeast TBP in the above-described assay for TAF binding to TBP. Thus, a subset of active compounds are identified that selectively interfere with yeast, and not mammalian, TAF-145 function, i.e., that are fungal-specific.

5 **B. HAT activity**

To each well of a microtiter dish is added 50 µl of a solution containing purified *C. albicans* TAF-145, buffer B (50 mM Tris-HCl pH8.0, 10%(v/v) glycerol, 1 mM DTT, 1 mM PMSF, 0.1 mM EDTA) and 25 µg of calf thymus histones (Sigma). Small molecules, whether purified or present in natural or synthetic mixtures, are introduced into the assay at concentrations ranging from about 20 to about 200 µM, and appropriate solvent controls are also performed.

The reactions are initiated by the addition of [³H]acetyl-CoA (100 nCi; 6.1 Ci/mmol; ICN) to a final concentration of 0.5 µM and are terminated after 10 minutes by spotting the entire mixture onto Whatman P81 filters. [³H]-Acetate incorporation is determined by liquid scintillation and nonspecific counts are subtracted. Compounds that inhibit [³H]-Acetate incorporation by at least about 25% are identified.

Compounds identified as described above are then tested for their ability to inhibit TBP-dependent transcription in a mammalian cell-free system. Alternatively, human TAF-145 is used in place of *Candida* TAF-145 in the above-described assay for HAT activity. Thus, a subset of active compounds are identified that selectively interfere with yeast, and not mammalian, TAF-145 function, i.e., that are fungal-specific.

Example 4: TAF Protein expression and purification for antibody production

Recombinant clones encoding for the HAT domain of the TAF protein, pFL 107 (*C. albicans* TAF 145 aa 339-766 in pET19b) [Figs. 8A, 8B and SEQ ID NO:7], pFL 110 (*S. cerevisiae* TAF 145 aa 354-835 in pET23a) [Figs. 8A, 8B and SEQ ID NO:8] and, pFl 123 (human TAF 250 aa 433-974 in pET23a) [Figs. 8A, 8B and SEQ ID NO:10] were each seeded into 10 ml of Luria Bertani (LB) broth supplemented with ampicillin (100 µg/ml) and grown overnight shaking at 37°C. The overnight cultures were seeded into 500 ml of LB/amp and grown shaking at 37°C. to mid-late log phase (O.D. of 0.6-0.8 at 600 nm). The cells were induced by the

- 5 addition of 0.4 M isopropyl β -D-thiogalactopyranoside (IPTG) (Sigma Chemical Co. St. Louis MO) and allowed to express the TAF proteins for 2 hours. The cells were harvested by centrifugation at 10,000 x g at 4°C. for 20 minutes and resuspended in Tris buffer (50mM Tris-HCL, pH. 8.0). The cells were lysed by sonication 3 x 3 minutes on ice using a Branson sonifier 250 (Branson Ultrasonics Corp., Danbury
- 10 CT) and the soluble portion was separated from the insoluble portion by centrifugation. The insoluble portion was solubilized by adding 3 ml of urea buffer (8 M urea) and incubated for 30 minutes to 2 hours at 37°C. The solubilized proteins were separated from cellular debris by centrifugation 10,000 x g for 20 minutes. SDS-PAGE sample buffer (0.5 M 2-mercapto-ethanol, 10% [wt./vol.] Sodium dodecyl
- 15 sulfate (SDS), 50% Glycerol, 0.5% Bromophenol blue) was added to the extracts and the samples boiled for 5 minutes.

- The solubilized proteins from *C. albicans* (Ca), *S. cerevisiae* (Sc) and the human (Hu) TAF proteins were loaded onto a 12% SDS-polyacrylamide gel (3mm in thickness). The samples were electrophoresed until the bromphenol blue dye front
- 20 ran to the bottom of the gels. The proteins were visualized by staining the gels with Coomassie blue stain (10% acetic acid, .25% Coomassie Brilliant Blue) and destained overnight with destain (7% acetic acid, 30% methanol). Destain was

5 removed from the gels and the gels were washed 3 x for 10 minutes in deionized water.

For purification of the *C. albicans*, *S. cerevisiae*, and the human TAF proteins. The protein bands were cut from the gel and electroeluted using a Centrilutor device (Amicon Inc., Beverly MA) according to the manufacturer's instructions. The
10 purity of the electroeluted proteins was determined on a coomassie stained 12 % SDS-PAGE gel (Bio-Rad, Hercules CA) (Figure 10B). Each TAF protein band was discrete and free of other contaminating proteins.

The purified protein against the *C. albicans* TAF145 aa 339-766 [SEQ ID NO:7] was then injected into rabbits to produce polyclonal antibodies (see
15 Example 5).

Example 5: Production of antibodies against *C. albicans* TAF145 HAT domain

Polyclonal antibodies directed against a portion of the HAT domain of
20 *C. albicans* TAF145 (aa 339-766) [SEQ ID NO: 7] were generated by injection of purified recombinant protein into rabbits (Robert Sargeant, Ramona CA). Sera from these rabbits were tested for their ability to recognize recombinant CaTAF145 HAT domain in an ELISA assay according to the following method:

1. Varying amounts of CaTAF145 HAT domain were aliquotted in 50
25 mM Borate, pH8.5, were added to Immulon 4 plates, were incubated for 25°C for 1 hour, and the plates were then washed.

2. The plates were then blocked with 200 μ l of a 5% milk solution in a Tris buffer (TBST)

TBST = 10 mM Tris, pH 7.5
30 150 mM NaCl
0.5% Tween-20

The plates were incubated for 30 minutes at 25°C, and were then washed.

5 3. The washed plates were then incubated with rabbit antisera at multiple dilutions (1:500, 1:1000, 1:2000, 1:4000) in TBST(5% milk) for 1 hour at 25°C, and were then washed.

 4. The plates were further incubated with a 1:1000 dilution of APGAR (Alkaline Phosphatase Conjugated Goat-anti-Rabbit polyclonal antibody Calbiochem)
10 in TBST/5% milk. The secondary antibody was incubated for 1 hour at 25°C, and the plates were again washed.

 5. The plates were then incubated with 2 mg/ml PNPP (para-nitro-phenylphosphate) in a 1X diethanolamine buffer (Pierce) for approximately 20 minutes. Once color developed, the plates were read at OD 405 nm

15 The ELISA assay data for the four dilutions tested is graphically represented in Figure 11.

Example 6: Expression of Human and *C. albicans* TAF proteins in Baculovirus

20 The full-length *C. albicans* TAF145 gene and a portion of the human TAF250 gene (aa 1-1218) [SEQ ID NO: 22] were PCR amplified and subcloned into pFastBac1 (Life Technologies Inc., Gaithersburg MD) to create pFL139 (*C. albicans*) and pFL149 (human), respectively. Oligonucleotide sequences (SEQ ID NO: 23- SEQ ID NO:28) are listed in the Table 5 below.

25

TABLE 5

Oligonucleotide	Organism	Sequence	Direction	Corresponding amino acids and restriction sites created	SEQ ID NO:
pCaHAT15	<i>C. albicans</i>	ACGCGTCGACATGC ATCATCATCATCAT CATATGGAGGATCT ACCCAGGGAT	Forward	amino acid 1 to ... 6xHis Tag and <i>SalI</i> site.	23
pCaHAT12	<i>C. albicans</i>	ATAGTTAGCGGCCG CACACTGCTGGTGT CAACCAACAA	Reverse	amino acid 1161 <i>NotI</i>	24
pHuHAT11	Human	ACGCGTCGACATGC ATCATCATCATCAT CATATGGGACCCGG CTGCGATTG	Forward	amino acid 1 to... 6xHis Tag and <i>SalI</i> site.	25
pHuHAT12	Human	GTTGCTCTGCAGCT ATCATGCTATAATA AGC	Reverse	amino acid 894 <i>PstI</i>	26
pHuHAT13	Human	TGATAGCTGCAGAG CAACGACTGAAGG ATGC	Forward	amino acid 895 <i>PstI</i>	27
pHuHAT15	Human	CCGGTACCTTCCCG ATGTTGTTTCATCAA AAAG	Reverse	amino acid 1218 <i>KpnI</i>	28

All oligonucleotides are written in the 5' to 3' direction.

Restriction enzyme sites are underlined.

pFL149 was created in two steps: the 2.7 kb PCR product created by oligonucleotides pHuHAT11 and pHuHAT12 was inserted into pFastBac1 to create pFL141 followed by insertion of the 0.9 kb PCR product created by oligonucleotides pHuHAT13 and pHuHAT15. Bacmid DNA was generated by transforming pFL139 and pFL149 into *E. coli* DH10Bac and selecting on Luria Agar plates containing 50 mg/ml kanamycin, 7 mg/ml gentamicin, 10 mg/ml tetracycline, 100 mg/ml X-gal and, 40 mg/ml IPTG. White colonies were selected for the isolation of recombinant bacmid DNA. Bacmid DNA was isolated according to the BAC-To-BAC Baculovirus Expression Systems Instruction Manual (Life Technologies Inc., Gaithersburg MD).

The preparation of bacmid DNA and transfection of SF-9 cells was done using the Bac-to-Bac™ (Life Technologies Inc., Gaithersburg MD) expression system according to the instruction manual. 5 µl of bacmid mini-prep DNA

5 (recombinant and wild type) and 6 μ l of Cellfectin (Life Technologies Inc., Gaithersburg MD) reagent were each diluted into 100 μ l of grace's insect medium supplemented with L-glutamine (Invitrogen Corp., San Diego CA). Each solution was mixed by gently inverting several times. The bacmid DNA and cellfectin solutions were combined and allowed to incubate for 30 minutes at rt. 0.8 ml of
10 grace's minimal media was added to the complex and gently mixed by inverting.

9x10⁵ sf9 cells were seeded into each well (35mm) of a 6 well plate and allowed to attach for 1 hour at room temperature. The cells were washed 2 x with 2 ml of Grace's minimal media. After the final wash the cells were transfected with bacmid DNA-Cellfectin complex for 6 hours rocking at rt. The trasfection media was
15 removed and 2 ml of Ex-cell™ 420 serum-free insect media (JRH Biosciences, Lenexa KS) was added to each well and incubated for 72 hours at 27°C. After 72 hours the transfected cells were removed from the plates and harvested by low speed centrifugation. The supernatant fluid containing baculovirus was removed and stored at 4°C.

20 1 x 10⁶ High-Five cells, available from Invitrogen, Carlsbad, CA, were seeded in each well of a 6 well plate and allowed to attach for 1 hour at room temperature. The media was removed and the cells infected with 100 μ l of a 1:10 dilution of transfection baculovirus supernatant. The cells were rocked for 1 hour at rt. The infected cells were supplemented with 2 ml of Ex-Cell media and incubated for
25 72 hours at 27°C. After 72 hours the cells were collected by washing the wells several times with media and the cells pelleted by low speed centrifugation. The supernatant media was removed and the cells resuspended in phosphate-buffered saline (PBS), pH 7.5.

Coomassie stain and Western blot analysis was used to examine the
30 expression of recombinant TAF protein in Baculovirus (Figure 12). 5 μ l of 5x SDS-PAGE sample buffer was added to 10 μ l of the cell suspensions and the samples boiled for 5 minutes. Cellular debris was removed by centrifugation and the proteins (15 μ l) separated on 12% SDS-PAGE gels (Bio-Rad, Hercules CA). The gels were

5 placed in a mini Trans-Blot apparatus (Bio-Rad, Hercules CA) and the proteins were electrophoretically transferred to nitrocellulose membranes. The membranes were blocked for 10 minutes with blocking buffer (5% skim milk, 20 mM Tris, 0.2 M NaCl, 0.1% Tween-20, pH 7.5). Rabbit polyclonal anti-*C. albicans* TAF 145 antibodies were used to probe the electroblotted infected high-five cell extracts. *C.*
10 *albicans* TAF 145 [SEQ ID NO: 2] and Human TAF 250 (aa 1-1218) [SEQ ID NO: 22] proteins were detected using goat anti-rabbit antibodies conjugated with HRP and ECL chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire England). Western Blot analysis illustrated that the Human (Figure 12; Lane 1) and the *C.*
15 *albicans* (Figure 12; Lane 2) HAT domains of the TAF protein were expressed in the Baculovirus system and the proteins were of the predicted size, 145 kDa.

Example 7: Deletion analysis of *C. albicans* TAF145

20 Deletion analysis of *C. albicans* TAF145 was used to establish if CaTAF145 is essential for cell viability. In wild-type *C. albicans*, CaTAF145 is encoded by two alleles (Figure 13B; lane 1).

Homologous recombination was used to integrate two plasmids into the yeast genome. For a schematic diagram illustrating the homologous
25 recombination see Figure 13A. Each of the plasmids contain TAF145 interrupted by selectable markers as follows: ADE (pSIK11) and URA3 (pFL125).

Construction of plasmids pSIK11 and pFL125

pSIK11 was constructed in the following manner:

30 1) 460 bp CaTAF145 upstream region was PCR amplified with primers TAF145p32f (ACGCGTCGACATCCAAGTTCAAGTTGTCTG) [SEQ ID NO:29] and TAF145p33r (CGCGGATCCGCGCTGCAGTTTTTCACATCTTCTT CT TCTGCCA) [SEQ ID NO:30];

- 5 2) 518 bp CaTAF145 downstream region was PCR amplified with primers
TAF145p34f (AAAACTGCAGCGCGGATCC GCGT GCA GGTGA
CGTTATTGGA) [SEQ ID NO:31] and TAF145p35r (ATAGTTTAGGCGGCCGCC
TTGTGACAA GAAGTGACAC) [SEQ ID NO:32];
- 10 3) PCR products from steps 1 and 2 were used as templates and primers TAF145p32f
and TAF145p35r were used to generate a single piece of DNA by PCR that contains
the upstream and downstream region of CaTAF145;
- 15 4) the PCR product from step 3 was cloned into Sall-NotI sites of pBlueScript
(Stratagene, La Jolla, CA);
- 5) ADE2 gene was PCR amplified and inserted into PstI-BamHI sites of the plasmid
created in step 5.
- 20 pFL125 was constructed similarly to pSIK11 except that in step 5, hisG CaURA3
hisG (Fonzi and Irwin, 1993) was inserted into PstI-BamHI sites.
- Yeast medium was prepared as described (Guthrie and Fink, 1991).
Transformation of *C. albicans* was performed using a lithium acetate procedure
(Schiestl and Gietz, 1989). To create a single disruption of TAF145 in *C. albicans*
25 strain CAI8 (Fonzi and Irwin, 1993), cells were transformed with plasmid pSIK11
(Δ Cataf145::ADE2) digested with Sall/NotI and ADE+ prototrophs were selected.
Integration of the Δ Cataf145::ADE2 cassette at TAF145 was verified by Southern
Blotting and using 32 P- labeled pSIK11 as a probe (Figure 13B; Lane 2). These singly
disrupted strains (CSIK1) were selected for further analysis and then an attempt was
30 made to delete the second TAF145 allele.
- The singly disrupted strain CSIK1 (TAF145/ Δ Cataf145::ADE2) was
transformed with pFL125 (Δ Cataf145::URA3) digested with Sall/NotI and Ade+
Ura+ transformants were selected. As a control, to determine if pF125

5 (ΔCataf145::URA3) was capable of integrating into the yeast genome, CAI8 was transformed with Sall/NotI digested pFL125 (ΔCataf145::URA3) and Ura⁺ transformants were selected. Analysis of a Ura⁺ transformant is shown in Figure 13B, lane 3.

Ura⁺ Ade⁺ transformants were screened by Southern blot for the
10 presence of both integrated cassettes (Figure 13B; Lanes 4-10). In 20 out of 20 transformants, the wildtype TAF145 band was still present, indicating that it was not possible to select for transformants with a deletion in the second copy of the TAF145 allele and that TAF145 is essential for cell viability. Figure 13B displays the results of Southern blot analysis for 7 of the 20 transformants. Each transformant selected
15 contained a deletion in only one copy of the TAF145 allele and only one selectable marker, either Ura or Ade but not both. This indicates that TAF145 is essential for cell viability.

References for Example 7

20 Fonzi, W.A., and Irwin, M.Y. (1993). Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134, 717-728.

Guthrie, C., and Fink, G.R. eds. (1991). Guide to Yeast Genetics and Molecular Biology. Academic Press, New York.

25

Schiestl, R.H. and Gietz, R.D. (1989). High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* 16, 339-346.

30

Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed disclosure. All such modifications are within the full extended scope of the appended claims. All patents and references mentioned in this application are hereby incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid having the sequence depicted in Figure 3, SEQ ID NO: 1.

2. An isolated nucleic acid that hybridize to a nucleic acid as defined in claim 1 under stringent hybridization conditions.

3. A nucleic acid vector comprising a nucleic acid as defined in claim 1 operably linked to a transcription regulatory element.

4. A cell comprising a vector as defined in claim 3.

5. A cell as defined in claim 4, wherein said cell is a member selected from the group consisting of bacterial, fungal, insect, and mammalian cells.

6. A method for producing a polypeptide, which comprises:
(i) culturing a cell as defined in claim 5 under conditions suitable for the expression of *C. albicans* TAF 145 polypeptide; and
(ii) recovering said polypeptide from said culture.

7. An isolated polypeptide having the amino acid sequence depicted in Figure 3, SEQ ID NO:2.

8. A fragment of a polypeptide as defined in claim 7 which inhibits the interaction of said polypeptide and TATA-box Binding Protein (TBP).

9. A method for inhibiting fungal transcription in a *C. albicans* cell comprising contacting said cell with an agent that selectively interferes with the

1 interaction of the polypeptide of claim 7 and TATA Binding Protein (TBP) .

1 10. An antibody that specifically recognizes *C. albicans* TAF 145
2 polypeptide.

1 11. A method for rapid, large-scale screening to identify a ligand that
2 binds to a *C. albicans* TAF 145 protein from a plurality of test compounds not known to
3 bind *C. albicans* TAF 145 protein, said *C. albicans* TAF 145 protein having been
4 incubated in the presence and absence of a plurality of test compounds under conditions
5 sufficient to unfold *C. albicans* TAF 145 protein in the absence of test compounds, which
6 comprises:

7 detecting an increase or a decrease in the amount of *C. albicans* TAF 145
8 protein in the folded state; and

9 determining that said test compound is a ligand that binds to said *C.*
10 *albicans* TAF 145 protein if there is detected an increase or a decrease in the amount of
11 said *C. albicans* TAF 145 protein in the folded state in the presence of said test
12 compound.

1 12. A method for rapid, large-scale screening to identify a ligand that
2 binds to a *C. albicans* TAF 145 protein from a plurality of test ligands not known to bind
3 to said *C. albicans* TAF 145 protein, said *C. albicans* TAF 145 protein having been
4 incubated in the presence and absence of a plurality of test ligands which comprises:

5 subjecting said *C. albicans* TAF 145 protein to unfolding conditions;

6 detecting an increase or a decrease in the amount of *C. albicans* TAF 145
7 protein in the folded state; and

8 determining that said test compound is a ligand that binds to said *C.*
9 *albicans* TAF 145 protein if there is detected an increase or a decrease in the amount of
10 said *C. albicans* TAF 145 protein in the folded state in the presence of said test
11 compound.

1
2
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4
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6
7
8
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FIG. 1A

A

△

1

FIG. 1C

818 PTGCGEGGFSFLKLTSMKGG S.cerevisiae
 744 PTGCGEGGSEIRISMKGG S.pombe
 1002 PTGCGEGGFSYVVRVFNKPTQTKEEQEISQP-KRISVTGTADLRRLPLQRAKEELLRQFKVPPEEEIKKLSRWEEV Drosophila
 956 PTGCGEGGFSYVVKIPNKPQTQQKDDKLEPQPVKKTVITGTDADLRRLSLKNAAQQLLRKEGYPEEEIKKLSRWEEV Human
 839 - - - - -SGSPSSNNSSNNKKGTNTHSYNVAQQQKAYDEEIAKIWYTHTKSSLSISNP S.cerevisiae
 765 - - - - -AGETADDDKPEPQTK -NAHAYNVAQQQRAYEEETIRIWNAAQKRGLSISNN S.pombe
 1071 IDVVRTLS TEKAKAGEEGMDKFSRGNRFSIAEHQERYKEECQRIFDLQNRVLA SEVLS TD EAESSIASIEE Drosophila
 1026 IDVYRTMSTEQARSCEGPMSSKEARCSRFSVAEHQERYKEECQRIFDLQNKVLS TEVLS TD T DSSSAE D Human
 889 - FEEMTNPDEI - - - - -NQINK - - - - -HVKTDRDD - - - - -K - S.cerevisiae
 812 - -LEELAKKYG I - - - - -NSIHDDYVESNEETIR EE - - - - -TPS S.pombe
 1141 SDLEELGKNLENMLSNKKTSQLSREREELERQELLRQLDEEHGGPSSGSGAKGAKGKD DPGQQMLA INN Drosophila
 1095 SDFEEMGKNLENM LQNKKTSQLSREREELERQELQRMLAAGSAASGN - - - - -NHRDDDTASVTS LNSSA Human
 913 - -KILKIVRKKRDENG IIRQQTIFIRDPRIQGYIKIK EQDKEDVNKL EEDTSKINNLEELKQKKLLO S.cerevisiae
 843 - -DKVLRIVRLYRDKNGLERKQ ETIHDP IIVIHAYLK -KRR EID EQSTAL DAVVPTGDEAID -RRNRRLLE S.pombe
 1211 QGRILRIITRFRGNDGKEYTRIVETVRRQPVIDAYIKIRITTKDEQFIKQFATLDE - -QKKEEMKRIEKKRRIQ Drosophila
 1161 TGRCLKIYRIFRDEEGKEYVBCETVRKPAVIDAYVRIRITTKDEEFIRKFA LFDE - -QHREEMRKE RRRIO Human
 981 LELAINLER SQRRRAARQNSKRRNG - - - - -GATRTE NSVDN GSDLAGVT D GKAARK GN TTR - - - -R S.cerevisiae
 911 QELAKSQKNWERRRARIHAAK - - - - - - - -EGINNG - - - - -KPTTR - - - -K S.pombe
 1279 EQLRIRIKRNQERERLAQLAQNQKLPQPGGMP TSLGDPKSSGGHSHKERD SGYKEVSPSRKKFKLKPD LK LK Drosophila
 1229 EQLRIRLKRNQEKEL - - - - -KGPPEKKP - - - - -KKMKERPD LK LK Human
 1038 CATCGQIGHIRTNNK SICPMYSSSKDNPIASP - - - - -K S.cerevisiae
 947 CSNCGQVGHMKTNKTCPIFGGRPEGGLATMLDKN S.pombe
 1349 CGACGQVGHMRTNNKACPLYSGMQSSL SQ - - - - -S Drosophila
 1264 CGACGAI GHMRTNNKFCPLY - - - - -YQ TNA PP - - - - -S Human

FIG. 2A

<i>S. cerevisiae</i>	P G P N S R	D P T G C G E G	G H I R T N K
<i>S. pombe</i>	P G P H S R	D P T G I G E G	G H M K T N K
<i>Drosophila</i>	P G P N S K	D P T G C G E G	G H M R T N K
Human	P G P N S K	D P T G C G E G	G H M R T N K

TAF145p1F	5' CC(A/T)GG(A/T)CC(A/T)AA(C/T)TCIA(A/G)(A/G)	
TAF145p2F		5' GA(C/T)CC(A/T)AC(T/C/A)GG(A/T)TGTGG(A/T)GAAGG
TAF145p3R	CT(G/A)GG(T/A)TGACCC(T/A)ACACCC(T/A)CTTCC	5'
TAF145p4R		CC(A/T)GT(A/G)TAATC(T/C)AC(T/C/A)TT(G/A)TT 5'

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FIG. 2B

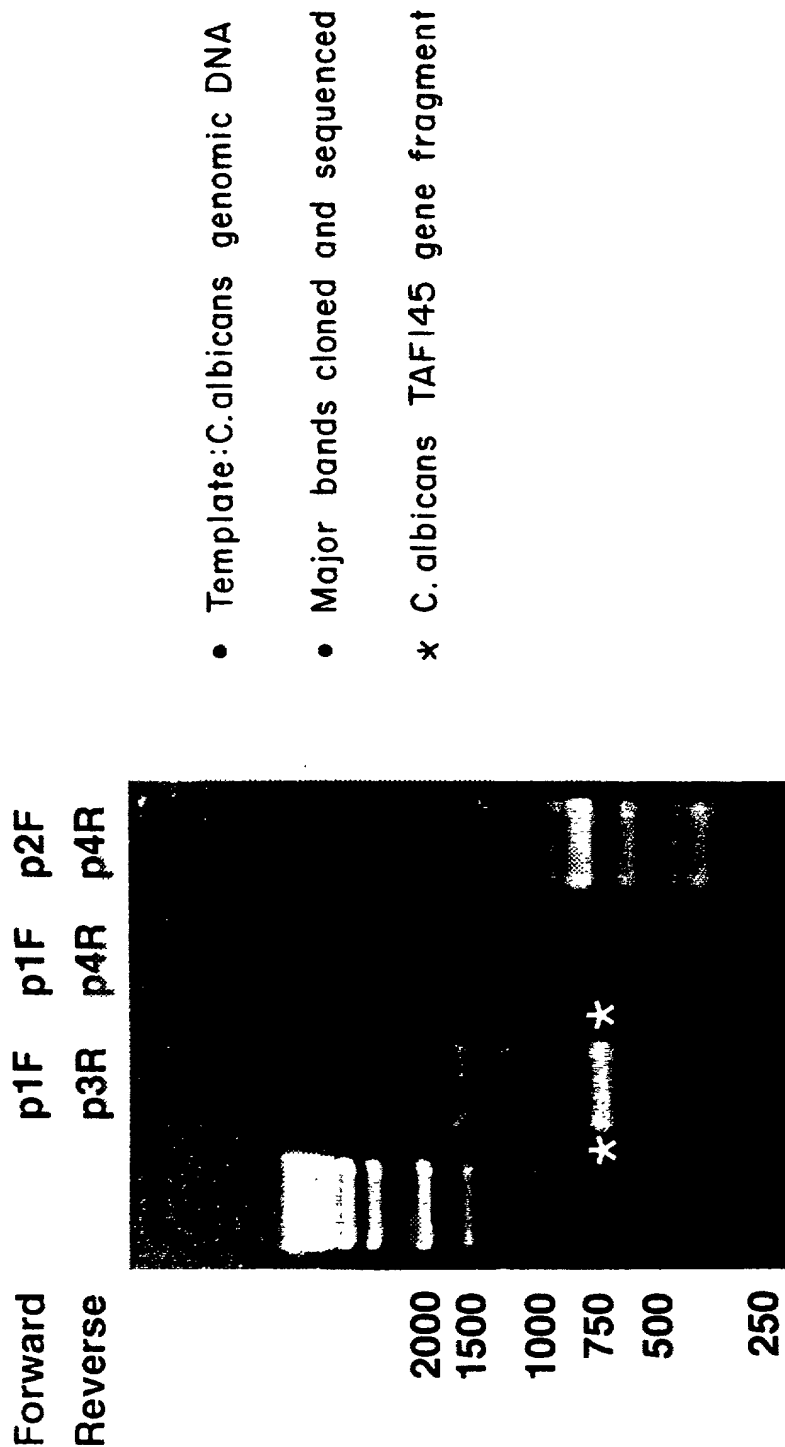


FIG. 3A

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1 aaaaaaaaaaagctccccgaaaaaactgaaacccaacaaacaaaaaagtaaaacaaacctctacaaaactcttatat 80
81 tatatcccttggttacatttatatatacgccaacaaacgaaggataatacacatttatgtatgataatgccagaagtcacacataaa 160
161 tcaaacatatagaagaagatgaagacaaaagcatacaatcaatatgtaaatagtagtactgatattccagatgatcaaaattat 240
241 agaatcatatttcagaacgaaaaaactgcccatgctgaagatgccatcgattatgaagatatagacgaattggcagaagaa 320
321 gaagatgtg ATG GAG GAT CTA CCC AGG GAT GAG GCA ATA AAT GGA TTA AAC TCA AAC AAC AAC 380
1 M E D L P R D E A I N G L N S N N 17
381 AAC AAC AAC CAC GAT AAA GAT GAT GAT GAC GAC GAT GAA TTC AAT AGG TTA CTT CAA GAA 440
18 N N N H D K D D D D D E F N R L L Q E 37
441 GGG CAG CCT GAA TTG ACA AAT GAT GAA GAA ATG GCA GCT CAA GCT GCT GCT GAA TCT CAA 500
38 G Q P E L T N D E E M A A Q A A A E S Q 57
501 TTT GAT GCT TTG TTT GGA AAC TCT AAT GAT AGT AGT AAT ATT AGC CAC CAT GAT CAT 560
58 F D A L F G N S N D F D S N I S H H D H 77
561 ATG GGC GGG GAC AGT AAT GGT ATT ATT GAT GAT AAT CAC CAC AGC AGT GTA AAC GAC CAC 620
78 M G G D S N G I I D D N H H S S V N D H 97
621 GAT GGT TTA TTC AAC AAT TTA GGA AAT GGT AAT CAT TTG CTA GAT GAT GAT AAT GAT GGC 680
98 D G L F N N L G N G N H L L D D D N D G 117
681 TTG AAT GAT TTA GGT GAA CTA TTT GAT GAT CAA CAA GAG GAC AGC AAT GTT ATC AAC ACT 740
118 L N D L G E L F D D Q Q E D S N V I N T 137
A _____ A

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FIG. 3B

A																					B																				
741	AAG	AAG	CAT	AAG	CTA	GAT	GAC	GAT	AGC	AAC	AAC	GAT	GGC	AAG	ACT	GCT	CAA	GAA	GAT	CAA	800																				
138	K	K	H	K	L	D	D	D	S	N	N	D	G	K	T	A	Q	E	D	Q	157																				
801	AAA	GAG	AAA	GAA	AAT	AAA	CGA	CAA	TTG	AAA	CGG	CAA	AAA	CTA	CAA	AAG	ATT	GTT	AAA	CAT	860																				
158	K	E	K	E	N	K	R	Q	L	K	R	Q	K	L	Q	K	I	V	K	H	177																				
861	CTT	GAG	AAG	GAA	CAA	ATC	AAA	CGA	AAT	ATA	AAA	TAT	TAT	TTC	CCT	ACT	TAT	TCA	AGA	CAT	920																				
178	L	E	K	E	Q	I	K	R	N	I	K	Y	Y	F	P	T	Y	S	R	H	197																				
921	AGA	CCA	TTT	AAT	TTT	CAC	AAA	TTT	TTT	TCA	CCA	AGT	CCT	CAG	TAT	TAC	CGT	TAT	CAA	AGA	980																				
198	R	P	F	N	F	H	K	F	F	S	P	S	P	Q	Y	Y	R	Y	Q	R	217																				
981	CCA	GCA	ATT	GCC	TTG	TCG	AAA	AAT	ATA	AAA	CCA	TTA	ATA	CCT	ACA	AAA	GTA	AAT	CTT	GAA	1040																				
218	P	A	I	A	L	S	K	N	I	K	P	L	I	P	T	K	V	N	L	E	237																				
1041	ATC	GAG	GTT	GAT	CAA	AAG	AAA	ATT	TTT	AAA	TTA	AGA	AGT	GCT	GAT	ACT	GCA	TCG	TTG	TCA	1100																				
238	I	E	V	D	Q	K	K	I	F	K	L	R	S	A	D	T	A	S	L	S	257																				
1101	CAC	GAA	GAC	AAA	AAT	GTC	ACC	AAT	ATT	ACT	CAA	GAT	GAC	TTG	GAT	TTT	ATC	AAA	AAT	TTA	1160																				
258	H	E	D	K	N	V	T	N	I	T	Q	D	D	L	D	F	I	K	N	L	277																				
1161	GAA	AGC	AAA	AGA	TCT	TCT	ATT	GAC	TCG	TTT	ATT	AAA	GAA	ATT	GAT	TAC	GTT	AAA	CGT	GAT	1220																				
278	E	S	K	R	S	S	I	D	S	F	I	K	E	I	D	Y	V	K	R	D	297																				
1221	TGG	ACT	AAT	TGC	GAC	AAG	TTT	GAT	CAT	TAT	TCG	AAA	GAT	TTA	GTT	CTA	TCT	ACC	ACT	GAT	1280																				
298	W	T	N	C	D	K	F	D	H	Y	S	K	D	L	V	L	S	T	T	D	317																				

FIG. 3C

B																		C			
1281	TGG	GAT	GAT	GCT	ATT	ATA	AAT	GCT	GGA	GAC	AAT	GAG	TAC	TCT	ATT	GTG	AAG	CCA	ATC	1340	
318	W	D	D	A	I	I	N	A	G	D	N	E	Y	S	I	V	K	P	I	337	
1341	AAT	GAG	CTT	TTG	CTC	AAC	AAT	CCC	TTG	GAC	AAT	AGT	AAA	CAG	AAT	AGA	CAA	AAA	ATC	GAG	1400
338	N	E	L	L	L	N	N	P	L	D	N	S	K	Q	N	R	Q	K	I	E	357
1401	AAT	GAC	AAT	ACT	ACC	AAC	AAC	TAT	AAC	CAA	AAC	AAT	AGT	AAT	GTC	CAA	GAT	GAG	GAG	GAG	1460
358	N	D	N	T	T	N	N	Y	N	Q	N	S	N	S	N	V	Q	D	E	E	377
1461	GAT	GAT	GAT	ATC	TTC	AAT	GGA	CAA	ATA	AAC	TTG	GAT	AAA	TTG	AAA	CTT	GAT	ATG	AAT	GAT	1520
378	D	D	D	I	F	N	G	Q	I	N	L	D	K	L	K	L	D	M	N	D	397
1521	CCT	AAC	TTG	TTA	TTT	GTT	CCT	AGT	AAA	AAA	GTC	GAT	GCT	ACC	AAA	TCA	GTG	GTT	CCA	AGT	1580
398	P	N	L	L	F	V	P	S	K	K	V	D	A	T	K	S	V	V	P	S	417
1581	ACA	GAT	AAA	TTA	TTA	GAA	TTA	AAG	TTT	AAC	ATA	TCT	AAC	GAT	CAA	GAG	TAT	GAA	TTA	TTG	1640
418	T	D	K	L	L	E	L	K	F	N	I	S	N	D	Q	E	Y	E	L	L	437
1641	AGA	AAG	AAT	TAC	AAC	ACC	AAA	CAA	AGA	TCT	CAA	TTG	AGT	AAT	CTT	AAT	ATT	GAA	CAT	TCA	1700
438	R	K	N	Y	N	T	K	Q	R	S	Q	L	S	N	L	N	I	E	H	S	457
1701	GTT	CCC	GCA	TTG	CGA	TTA	CAG	ACA	CCT	TAT	TAT	AAA	GTC	AAA	CTT	AGC	ACA	GAT	GAA	ACG	1760
458	V	P	A	L	R	L	Q	T	P	Y	Y	K	V	K	L	S	T	D	E	T	477
1761	AGA	TCA	TTT	CAT	CGA	CCA	GTG	TTT	AAT	GTC	AGA	CCT	GGT	ACA	TTG	GTG	AGC	TTT	TCT	AAA	1820
478	R	S	F	H	R	P	V	F	N	V	R	P	G	T	L	V	S	F	S	K	497
1821	TTG	AAG	TTG	CGG	AAG	CGG	AAA	AAA	GAC	AAG	GGG	AAA	TCT	TTG	CAA	CAG	ATT	TTT	TCC	AAA	1880
498	L	K	L	R	K	R	K	K	D	K	G	K	S	L	Q	Q	I	F	S	K	517
C																					

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FIG. 3E

2361 TTA GGA GTG CCA CGT ATT TCT GTG AAA GAT GTT TCC AAA CAT TTT CCT GAG CAT AGC GAT 2420
 678 L G V P R I S V K D V S K H F P E H S D 697
 2421 ATG CAA AAT CGA CAA AGA TTG AAA GAA TTT ATG GAA TAC CAA AGA CAA GGT GAG GAC CAA 2480
 698 M Q N R Q R L K E F M E Y Q R Q G E D Q 717
 2481 GGG TAT TGG AAA GTA AGA GGA TTA AAC GAT GTA ATT CCA GGA GAA GAG GAA ATT CGA ACT 2540
 718 G Y W K V R G L N D V I P G E E I R T 737
 2541 ATG ATT ACA CCA GAA GAT TCT TCA TTG ATG GAC ACA ATG CAA TTT GGA CAG CAA GTA CTA 2600
 738 M I T P E D S S L M D T M Q F G Q Q V L 757
 2601 GAT GAT AAT ATG GTG TTA TTC GGT GAG CAG AGC AGA CAA GAA TCA TCT AGA AGT AGA AAA 2660
 758 D D N M V L F G E Q S R Q E S S R S R K 777
 2661 GGT GAT AAA AGA GAG GAT TCC ATT GCG GAT GAT GCA GAA AAT GGA GAT GAT ATA AAT AAG 2720
 778 G D K R E D S I A D D A E N G D D I N K 797
 2721 GAT AAA GAG AAA GAG GTT GAG AAA GAA AAG GAA CAG GAA AGA GAA GAA GAG AAA GGG AAA 2780
 798 D K E K E V E K E Q E R E E E E K G K 817
 2781 GAT AAG GAA AAA GAC AAG GAC AAG GAA AAA GAC AAA ACC GAG AAG GAG AAA TCA AAG AAA 2840
 818 D K E K D K D K E K D K T E K E K S K K 837
 2841 TCA AAG GAG CAA GAC ACT GAA ATT GAT GTT GAA GAA TTG GCA CCA TGG AAC TTA TCG 2900
 838 S K E Q D T E I D V E E E L A P W N L S 857
 2901 AGA AAT TTT GTT ATC GCC AAT CAG ACC AAG ACA ATG CTA CAA TTG AAT GGT GAA GGT GAT 2960
 858 R N F V I A N Q T K T M L Q L N G E G D 877

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FIG. 3G

F _____

3501 CGT CGT AAA GCA TTG GAA GAA AAG TTG GCT AAT TTG GAG AAA CGG GCA AAA CAA AGC CGG 3560
1058 R R K A L E E K L A N L E K R A K Q S R 1077

3561 GCC AAA CCA CCA AAG GAT CTG ATC CAT GCA GCT GCT GCT GCG GCG GCA ACA ATT ATA 3620
1078 A K K P P K D L I H A A A A G A T I I 1097

3621 GAT GCT AAT ACT GTG ATG TTA CCA GAT GGA TCG TAT GTT ATT GGT GGT AAG GGT ATT GGT 3680
1098 D A N T V M L P D G S Y V I G G K G I G 1117

3681 AAG GGG AAA AGT CGA ACT CGT TGT AAA AAT TGT GGA GCT TAT GGA CAC ATT CGT ACT 3740
1118 K G K S R T R C K N C G A Y G H I R T 1137

3741 AAT GCA AAA TGT CCC TTA TAT AAG AAA ATG GTG CTT GGA ATC GAT GAT GAT TCA GCG GCA 3800
1138 N A K C P L Y K K M V L G I D D S A A 1157

3801 GTT GTT GGT TGA caccagcagtttagtgaggtagtgacggttattggagaaacacacatctaccgcagtaactcttga 3876
1158 V V G * 1161

3877 tacacagcgtatcagggaacagaatctggctgaagcgtgaccaatggcaatcaaaaatagatatgaacccagagatagga 3956

3957 gttactgagttgtcaaaaattagagtggaaatcagcaatgcatctttgtctcatcaaaacggacatgaacgagagtgtgatgat 4036

4037 gttattgtcagtaatatagggttagttacatttttaataatgacatatataaacaatgtaattattattatgcatttctatttgt 4116

FIG. 4A

C. albicans	MEDLPREAIINGLNSNNNHKDDDDDEFNRLLOEQOPELTINDEMAQAQAESQFDALFGNSNDFS	70
S. cerevisiae	GGDEGANSKDYTEHLPAVDFFEDELADDDDLPEESDANLHPAMMIMGAYDDNENGAVLGIDSN-SL	105
C. albicans	NLSHHDHMGDSNGLIIDNHSSVNDHGLENNLGNHLLDDNDGINDLGELFDDQQEDSNVINTKHH	140
S. cerevisiae	NMQLPEINGDLSQQFLEDDCGGTPATSNALFMGMDANEIHLATEIGVLDGSG-----ANEIGHSQLSIGGV	171
C. albicans	KLDDDSNNDGKTAQEDQKEKENKROLKQKLQKIVKHLEKEQIKRNIKYFPTYSRHPENFHKFFSPSP	210
S. cerevisiae	NGNDMSINGGFTMEPDMSDCKHKKA---TKLD-LINH-EKYLK---KY-FPDFEKGKILKWNKLIYRRS	232
C. albicans	QYRYQRPATAISKNIKPLIPITKANLEIEVDQKKIFKLRSADTASLSHEDKNVINITQDDLDFTKNLESK	280
S. cerevisiae	--VPYHMS-EISRVKPEMPLNLKFKVQDDKRLFNSRTISYVAPTYQGN--NLLQSNSSASRR-GLI	296
C. albicans	RSSIDSFIKEIDYVKRDWINCDKFDHYSKDLVLSITDMDDAIINAGDNEYSIVKPINELLINPLDNSK	350
S. cerevisiae	HVSIDELFP-IKEQQKRKIIDEKTISEDLIIATDWDQEKIINQGISSSTATLADSSMTFNLKFSGGYK	365

A_____A

FIG. 4B

A _____

C. albicans QNRQKIENNTINN 364
S.cerevisiae LKSLIEDVAEDWQW 379

C. albicans EDDIFNQIINLDK-LKLDNDENLLFVPSK-----KVDAKSVPSIDKLLLEKFNISNDQEYEL 436
S.cerevisiae DEDMIIDAKLKESKHAELNMDEKLLIMIEKINNLAQQKQQLDSSNLILPINETILQQKFNLSNDDKYQI 449

C. albicans LRKNYNIKQRSQLSNLTNIEHSVPALRLQTPYKVKLSIDEIRSFHRPVF---NVRREGILVSFSKLLKLRKRK 504
S.cerevisiae LKKTHTQTKVRSITISNLTNIQHSQPAINLQSPFYKVAVPYQQLRHFHRENFSGSHIRFGIKIVFSKLLKARKRK 519

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C. albicans KDKGKSLQQIFSKTSDLTVADIGNIILALEYSEQYPPILSNFGMGSKLINYYRKERENDISRPKAQIGEIH 574
S.cerevisiae RDKGKDVKESFSTSQDLTIGDIPAPVYLMYSEQTTPVALSKFGWANKLINYYRKANEQDILRPKLPVGEIH 589

C. albicans ILGVEDRSPFWNFGEVAPGDFVPTLYNNMVRAPIFKHINKPTDELLVKSQGAGSHQKFYLRGINENFVAVG 644
S.cerevisiae VLGVDKSPFWNFGEVFGHIVPTLYNNMIRAPVFKHDISGIDELLTKSSGFGISNRFYLRNINHLFVAVG 659

B _____

FIG. 4C

[illegible]

FIG. 5A

A_____A

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FIG. 5B

A		B	
458	VPA LR LQ T P Y Y K V K L S T D E T R S F H R P V F - - N V R P G T L V S F S K L K L R R R K K D K G K S L Q Q I F C. albicans		
471	QPA IN LQ S P E Y K V A V P R Y Q L R H E H R E N E G S H I R P G T K I V F S K L K A R R R K R D K G K D V K E S E S. cerevisiae		
393	AFA EK L T F P Y Y K T R L S K R A V R S Y H R P T M - - S F K P N A A I V F S P L I V R K R S K D K H K S E R E L I S. pombe		
516	S K T S D L T V A D T G N I I A L E Y S E Q Y P P I L S N F G M G S K L I N Y Y R K E R P N D T S R P K A Q I G E T H I C. albicans		
531	S T S Q D L T I G D T A P V Y L M E Y S E Q T P V A L S K E G M A N K L I N Y Y R K A N E Q D T L R P K L P V G E T H V S. cerevisiae		
451	P T T K E I T M G D T T H A I L V E F S E E H P A V L S N A G M A S R I V N Y Y R K K N E Q D E S R P K L E V G E S H V S. pombe		
576	L G V E D R S P F F W N F G E V A P G D F V P T L Y N N M V R A P I F K H D N K P T D F L L V K S Q G A G S H Q K F Y L R C. albicans		
591	L G V Q D K S P F F W N F G F V E P G H I V P T L Y N N M I R A P V F K H D I S G T D F L L T K S S G F G I S N R F Y L R S. cerevisiae		
511	L D V Q D R S P F F W N F G S V E P G E I T P T L Y N K M I R A P L F K H E V P T D F I L I R N S S S - Y G S K Y Y L K S. pombe		
636	G I N F N F A V G N T F P V E - V P A P H S R K V T N I S K N R L K M V V F R V M N S L G V P R I S V K D V S K H F P E C. albicans		
651	N I N H L F T V G Q T F P V E E I P G P N S R K V T S M K A T R L K M I I Y R I L N H N H S K A I S I D P I A K H F P D S. cerevisiae		
570	N I N H M F V S G Q T F P V T D V P G P H S R K V T T A S K N R L K M L V F R L I R R S P N G G L F I R Q L S K H F S D S. pombe		
695	H S D M Q N R Q R L K E F M E Y Q R Q G E D Q G Y W K V R G L N D V I P G E E E I R T M I T P E D S L M D T M Q F G Q C. albicans		
711	Q D Y G Q N R Q K V K E F M K Y Q R D G P E K G L W R L K D - D E K L L D N E A V K S L I T P E Q I S Q V E S M S Q G L S. cerevisiae		
630	Q N E M Q I R Q R L K E F M E Y K K K G D G P G Y W K L K S - N E V V P D E A G T R S M V S P E T V C L L E S M Q V G V S. pombe		
755	Q V L D D N M V L F G E Q S R Q E S S R S R K G D K R E D S I A D D A E N G D D I N K D K E V E K E Q E R E E C. albicans		
770	Q F Q E D N E A - Y N F D S K L K S - S. cerevisiae		
689	R Q L E D A - - - - - G Y G K T M D E I - S. pombe		
815	K G K D K E K D K E K D K T E K S K S K E Q D T E I D V E E E L A P W N L S R N F V I A N Q T K T M L Q L N G C. albicans		
787	- S. cerevisiae		
704	- S. pombe		

55

875	E G D P T G I G L G F S M L R A T Q K N P F K P L F T I P P E N I V P K S N - - - - A A I A H N Q Q K L Y E Q J E I K R U C. albicans
815	V I G D P T G C G E G F S F L K T S M K G G F V K S G S P S S N I N S S N K K I G T N T H S Y N V A Q Q K A Y D E E I A K S.cerevisiae
741	E G D P T G I G E G Y S F I R I S M K G G F K P A G E T A D D K P E P Q T K I - - N A H A Y N V A K I Q Q R A Y E E E I N R S.pombe
927	I W Y S Q R S S L V D H G E G T E S K L Q O I Y N E Y P P A D H E L Y L K N K L E Q D - - Q V Q Q Q Q Q D P S L Q A C. albicans
875	T W Y T H T K S L - - - - S I S N P F E E - - - - M T N P - - D E I N Q - - - - T S.cerevisiae
799	I W N A Q K R G L - - - - S I N N L - E E - - - - L A K K Y G I N S I H D - - - - D S.pombe
984	- - D Q Q Q Q Q Q Q - - - - N R V L R I T R R V R D E N G I V H R K V E F I H D P R L I R A Y V K R K K Q I E D E L C. albicans
902	- - N K H V K T D R D D - - - - K K I L K I V R K K R D E N G I I Q R Q T I F I R D P R V I Q G Y I K I K E Q D K E D V S.cerevisiae
828	Y V E S N E E T T R E E T P S S D K V L R I V R L Y R D K N G N L E R K Q E T I H D P I V I H A Y L K K R R E I D E Q - S.pombe
1038	L K N A D V D E I L P T N D K E L N K I R R K A L E E K L A N L E K R A K Q S R A K K P P K D L I H A A A A A G A T I I C. albicans
956	N K L L E E D T S K I N N L E E L E K - Q K K L L Q L E L A N L E K S Q R R A A R Q N S K R - - N G G A T R T E N S V S.cerevisiae
887	- - S T A L D A V V P T G D E A I D R R N R R L E Q E L A K S Q K N W E R R R A H A K E - - - - - S.pombe
1098	D A N T V M L P D G S Y V I G G K G I G K G K S R T R R C K N C G A Y G H I R T N A K C P L Y K K M V L G I D D S A I A C. albicans
1013	D N G S D L - - - A G V T D G K A A R N K G K N T T R R C A T C G Q I G H I R T N K S I C P M Y S S - - - - K D N P L A S.cerevisiae
932	- - - - - G I N L N G E G R - - - - K P P T T R K C S N C G Q V G H M K T N K I C P L F G R - - - - P E G L S.pombe
1158	V V - - - - G
1064	S P - - - - K
973	A T M L D K N

FIG. 6A

[illegible]

A

A

FIG. 6B

A

377 EDDDDIE NGQI --- NL--DKKKL DMND --- TNNLAQQK --KKVDAATKKVVV--PSTDXL--E C. albicans
 389 LKIESK--HAEL--NMNDEKLLLMIEK--TNNLAQQK--KKVDAATKKVVV--PSTDXL--E C. albicans
 390 FENIE DQDI ---GTSKVVL NND--PKLLQQLPKKEDSQRIFA--TNNLAQQK--KKVDAATKKVVV--PSTDXL--E C. albicans
 528 QVSKVPKPKVLTLDPNDENIILGIPDDIDPSIKINKSTGPPPKIKIPHPHVKKSKILLGKAGVINVLAEETPPPKSPDR Drosophila
 490 AMPRLLEPPVLTLDPNDENIILEIPDE---KEEATSNSPSIKESKKESSLKKSRIILLGKTGVIK--EPPQNNMSQPEVK Human
 424 LKFNISNDQEEYELLRKNYNTKQRSQLSNLNIEHSVIPALRLQTPYRKVKLLSTDETRSFHRP" F---NVRIPGTLVSIFS C. albicans
 437 QKFNLSNDDKYQILKKTHTKVRSTISN LNIOHSQPAINLQSPF YKVAVPRYQLRHEHRENF---GSHIRPGGIKIVFS S. cerevisiae
 359 WKFNISNDPAYEMLKQNHQSKVRNTLSQLAIEHAFAIEKLTFTFY YKTRLSKRAVRSYHRRPTM---S--FKDPAIIVFS S. pombe
 608 DPIENISNDITYT--PKTEPTLRKVGGINLIQHS TPVIVELRAPFVPITHMGPMMNVRRAIFHRPPLKKYSHGIPMAQSIHPHIVFP Drosophila
 503 DPMNLSNDEYY--PK--QQGLRGIFGGINIQHSIPAVELRQDFPITHMGPDIKLRQFHRPPLKKYSHGIPMAQSIHPHIVFP Human
 497 KILKLRKRKKDKGKSLQOI---FSKISDLTVADTGNIIALEYSEQYVPPILSNFGMGSKLINYYRKIERPNDTSRPKAQ C. albicans
 512 KILKARKRKRDKGKQVKE S---ESTSQDLTI GDTAPVYLM EYSEQTPVALSKFGMANKLINYYRKIA NEQDILRPKL P S. cerevisiae
 432 PLIVRKR'SKDKHKSEREL---IPTIKETIM GDTTHALIV EFS EEPH AVLSNAGMASRIVNY YRKKEQDES RPKLE S. pombe
 685 LKKTIAKAKQREVERIA SGGGDVFFMRNPEDLSGRDGDILVLAEECEEHDP LINQVGMCS KIKNY YKRKA EKSDSGPQDYV Drosophila
 639 LKHKIKKAKMREQERQASGGGGENFMRTPQDLTGKDGDLILA EYSEENGPLMMQVGMATKIKNY YKRKA EKSDSGPQDYV Human
 570 IGETHILGVEDRSFNNFGEVAPGDFVPTLYNNMVRAPVFKHDNKP TD FLLVKSQAGSHQXKFYLRGINFNAVGNTPPV C. albicans
 585 VGEIHLGVQDKSPFNNFGEVEPGHIVPTLYNNMIRAPVFKHDNKP TD FLLVKSQAGSHQXKFYLRGINFNAVGNTPPV S. cerevisiae
 505 VGESHVLDVQDRSPFNNFGEVEPGHIVPTLYNNMIRAPVFKHDNKP TD FLLVKSQAGSHQXKFYLRGINFNAVGNTPPV S. pombe
 765 YGEV--AFATSPF--LGLHPGQCIQAIENNMV RAPIYPHKMAHNDLIVIRTR--NNYWRISVMSIYIVGQECIPL Drosophila
 719 YGET--VYCHISPE--LGLHPGQCLLQAFENNLFRAPIY LHKMDETDLIVIRTR--QGYVIR ELVDIFVYVGOQC IPL Human
 650 E-VAPAHSRKVTNISKNRRLKMWVFRVM--NSLGVPRISVVKDVSXKHFPEHSDMQNRQLKEFMEYQRQIGEDQGYMKVVRGLN C. albicans
 665 EEIIPGPNRSRKVTSMKATRLKMIYRIL--NHNHSAKAIISIDPITAKHFPDQDYGQNRQKVKKEFMKYQRQIGEDQGYMKVVRGLN S. cerevisiae
 584 TDVPGPSSRKVTIASKNRRLKMWVFRIL--RSPNGGLFIRQLSKHFSIDOMEHQIRQLKEFMEYKKKGIDGPIGYMKVVRGLN S. pombe
 835 YIEVPGPNRSKRANFTTRDFLQVFIYRLFWKISRDPNRRIRMRMDIKQAFPIAHSES SIKRRLKQCADFKRTGMDSNWVVKP--E Drosophila
 789 FIEVPGPNRSKRANFTTRDFLQVFIYRLFWKISRDPNRRIRMRMDIKQAFPIAHSES SIKRRLKQCADFKRTGMDSNWVVKP--E Human
 727 DIVIPGEEFIRTMITPESSLMDTIMQFGQVILDDNMVLF
 742 EKLLDNEAVKSLITPEQISQVESHMSQGLQFQEDNEA-Y
 661 LEVVPDEAGTRSMVSPETVC LLESMSQVVRQLDAG--Y
 914 FRLPSEFEIRAMVSPQCCAYFSMIAAEQRLKIDAG--Y G
 868 FRLPTEFEIRAMVSPQCCAYFSMIAAEQRLKIDAG--Y

B

B

GECC

847	V	E	E	L	A	P	W	N	L	S	R	N	F	V	I	A	N	Q	T	K	I	M	L	Q	L	N	G	E	G	D	P	T	G	I	G	L	G	F	S	M	L	R	A	I	A	Q	K	I	---	---	---	---	C. albicans																							
787	L	E	E	N	L	P	W	N	I	T	K	N	F	I	N	S	I	Q	M	R	A	M	I	Q	I	H	G	V	G	D	P	T	G	C	G	E	G	F	S	F	L	K	T	S	M	K	I	---	---	---	---	S. cerevisiae																								
713	A	E	I	Q	L	L	A	P	W	I	T	T	R	N	F	I	N	A	I	G	K	A	M	I	L	F	I	G	E	G	D	P	T	G	I	G	E	G	F	S	E	I	S	I	M	K	I	---	---	---	---	S. pombe																								
972	E	V	K	V	A	P	W	N	T	T	R	A	Y	I	Q	A	M	R	K	C	L	L	O	L	S	G	P	A	D	P	T	G	C	G	E	G	F	S	Y	V	R	P	N	K	I	---	---	---	---	Drosophila																										
926	E	V	R	T	A	P	W	N	T	T	R	A	F	E	I	A	A	M	K	G	K	C	L	L	E	V	T	G	A	D	P	T	G	C	G	E	G	F	S	Y	V	K	I	P	N	K	I	---	---	---	---	Human																								
901	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C. albicans																										
841	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	S. cerevisiae																											
767	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	S. pombe																										
1050	E	L	L	R	Q	F	K	V	P	E	E	I	K	K	S	R	M	E	V	I	D	V	V	R	T	L	S	T	E	K	A	K	A	G	E	G	M	D	K	F	---	---	---	---	---	---	---	---	---	---	---	---	S. pombe																							
1005	Q	L	R	K	F	G	V	P	E	E	I	K	K	S	R	M	E	V	I	D	V	V	R	T	M	S	T	E	Q	A	R	S	G	E	G	P	M	S	K	F	A	R	G	S	R	F	S	A	E	H	Q	E	R	Y	K	E	E	C	Q	R	I	F	D	L	Q	N	R	V	L	A	S	E	V	L	S	Drosophila
937	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	C. albicans																
884	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	S. cerevisiae																
844	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	S. pombe																
1130	T	D	E	A	E	S	J	A	S	E	D	L	E	E	L	E	G	N	L	E	N	M	L	S	N	K	T	S	T	Q	L	S	R	E	E	L	E	R	Q	E	L	L	R	Q	L	D	E	E	H																											

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30°C

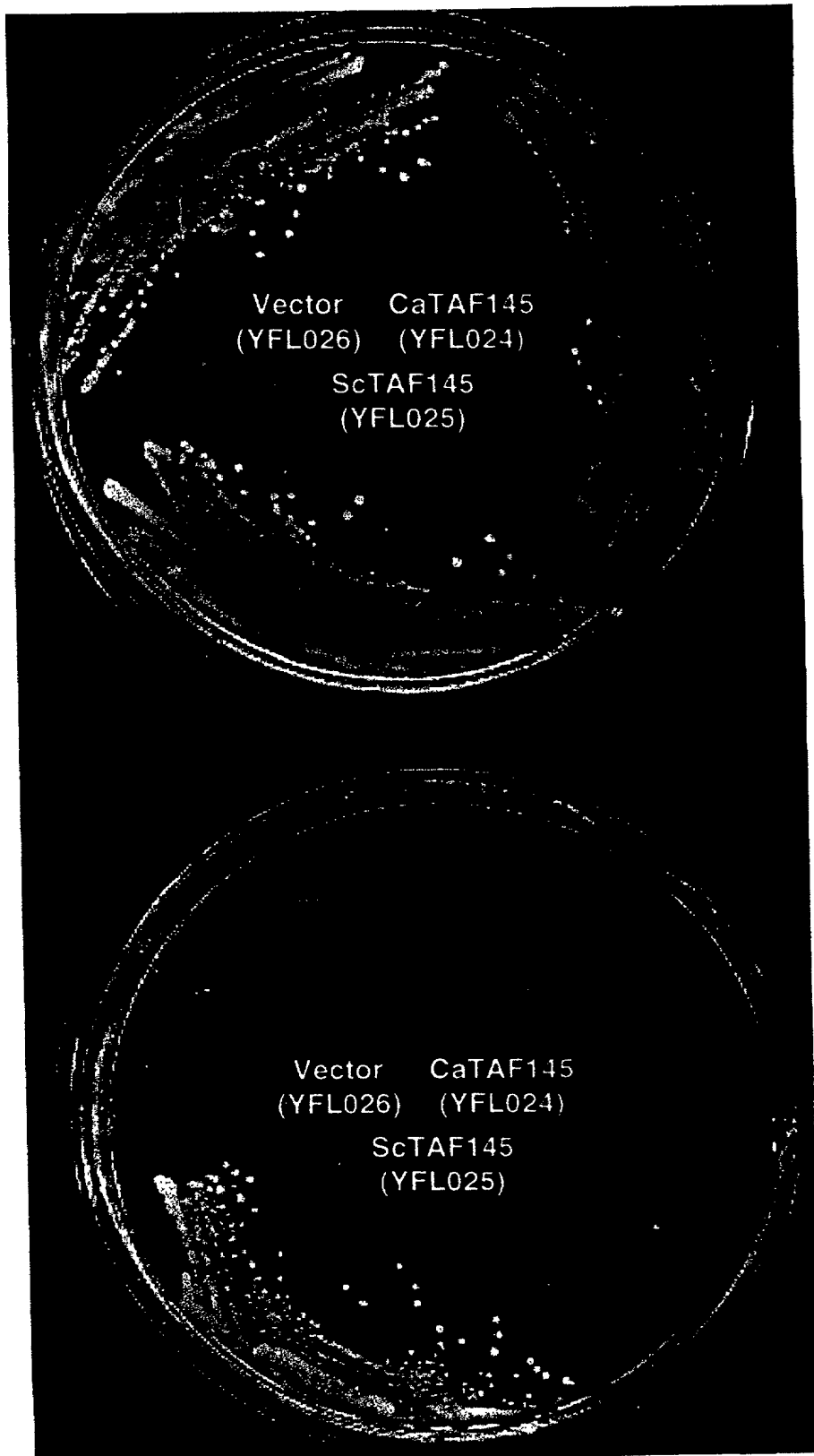


FIG. 7A

37°C

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SC-Leu

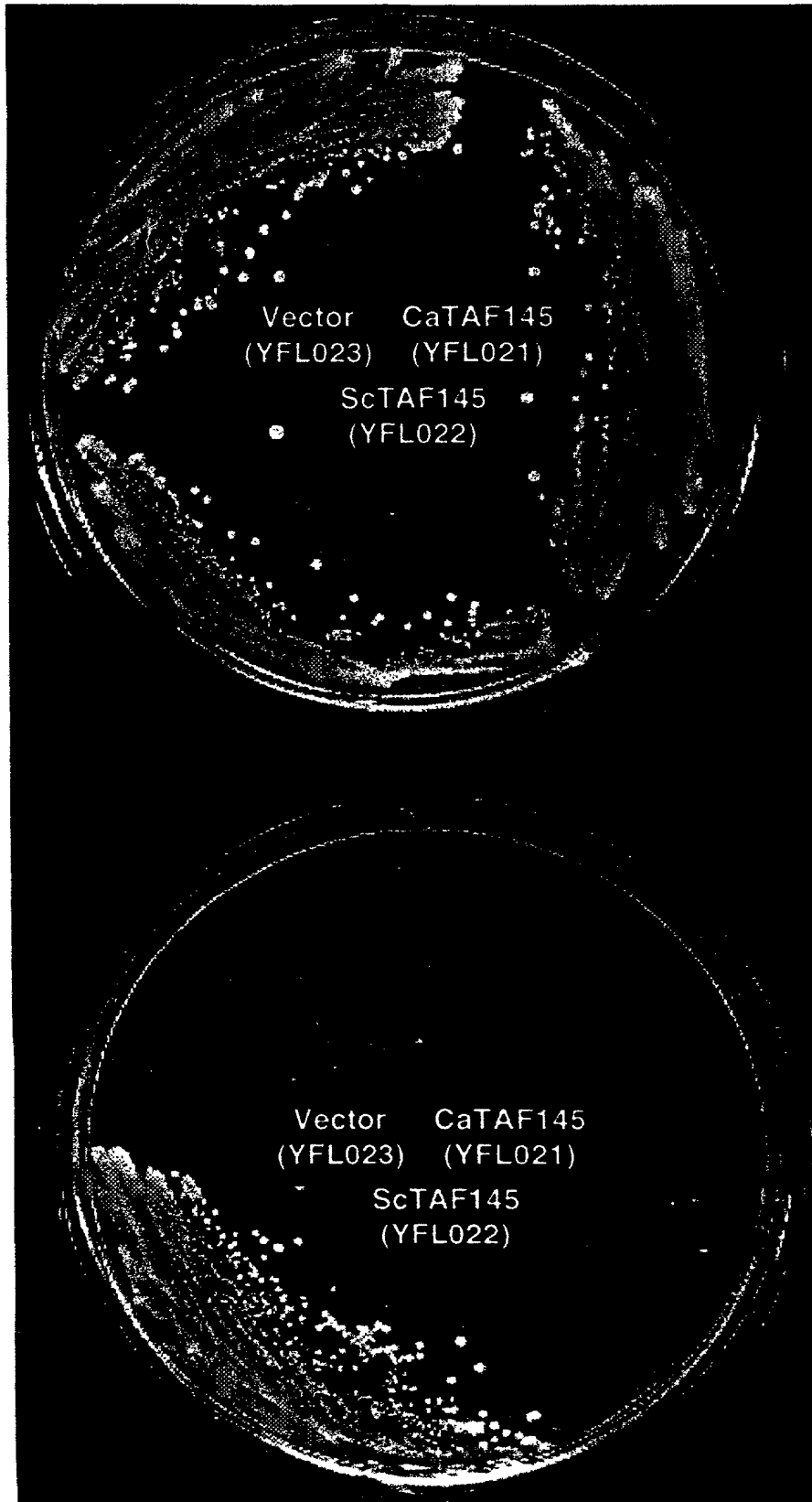


FIG. 7B

SC-Leu + 5-FOA

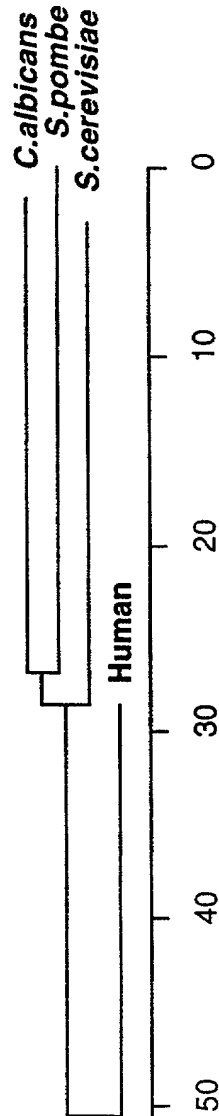
FIG. 8A

339	E L	- - -	L N N P L	D N S K Q N R Q	K I E N D N T	T N - - - Y - - -	- - -	- - -	N Q N C. albicans
354	- - -	- - -	M T P N L	K F S G G Y K L K S L	I E D V A E D	- - - W - - -	- - -	- - -	S. cerevisiae
288	- - -	- - -	V N K T N Q S	S F F I D K S L V D	- - -	- - -	- - -	- - -	S. pombe
432	S L	A G W L	P S S M T R N A M A Y	N V Q Q G F A A T L D D	D K P W	Y S I F P I D N E D L V Y G R	W	- - -	Human
369	N S	N V Q D E E E D	D I F N G Q I - - -	N L - - D K L K L	D M N D P N L L F V P S	- - - K K V	D A T	C. albicans	
381	E D	M I I D A K L K E S K	- H A E L - - -	N M N D E K L L L M	I E K T N N L A Q Q K	- - - Q Q L	D S S	S. cerevisiae	
305	- - -	- I D F A F D E N I F D G D T	- - - - -	G T S K V V L N L N D P K L L L Q P Q L	P K K E D S Q	- - -	- - -	S. pombe	
482	D N	I W D A Q A M P R L L E P P V L T L D P P	N D E N L I L E I P D E K E E A T S N S	P S K E S K K	Human	- - -	- - -	- - -	
412	K S	V V P S T D K L - L E L K F N I S N D	Q E Y E L L R K N Y - - -	- - -	- - -	- - -	N T K	C. albicans	
425	N L	I L P L N E T I - L Q Q K F N L S N D	D K K Y Q I L K K T H - - -	- - -	- - -	- - -	Q T K	S. cerevisiae	
346	R S	F A D T H Q R N S L A W K F N I S N D	P A Y E M L K Q N H - - -	- - -	- - -	- - -	Q S K	S. pombe	
532	E S	- - S L K K S R I L L G K T G V I K E E P Q Q N M S Q P E V K D P W N L S N D E Y Y P K Q Q G	Human	- - -	- - -	- - -	- - -	- - -	
445	Q R S Q	L S N L N I E H S V P A L R L Q T P Y Y K V K L S	T D E T R S F H R P - - -	- - -	- - -	- - -	V F -	N V C. albicans	
458	V R S T	I S N L N I Q H S Q P A I N L Q S P F Y K V A V P R Y Q L R H E H R E - - -	- - -	- - -	- - -	- - -	N F G	S H I S. cerevisiae	
380	V R N T	L S Q L A I E H A A F A E K L T F P Y Y K T R L S K R A V R S Y H R P - - -	- - -	- - -	- - -	- - -	T M S	- F S. pombe	
580	L R G T	F G G N I I Q H S I P A V E L R Q P F F P T H M G P I K L R Q F H R P L K K Y S F G A L S	Human	- - -	- - -	- - -	- - -	- - -	
488	R P G T	L V S F S K L K L R K R K K D K G K S L Q O I - - -	- - -	- - -	- - -	- - -	F S K I	S D L T V A D T G N I I C. albicans	
503	R P G T	K I V F S K L K A R K R K R D K G K D V K E S - - -	- - -	- - -	- - -	- - -	F S T S	Q D L T I G D T A P V Y S. cerevisiae	
423	K P N A	A I V F S P L I V R K R S K D K H K S E R E L - - -	- - -	- - -	- - -	- - -	I P I T	T K E I T M G D T T H A I S. pombe	
530	Q P G P	H S V Q P L L K H I K K K A K M R E Q E R Q A S G G G E M F E M R I P Q D L T G K D G D L I	Human	- - -	- - -	- - -	- - -	- - -	
531	A L	E Y S E Q Y P P I L S N F G M G S K L I N Y Y R K K E R P N D T S R P K A Q I G E T H I L G V E D	C. albicans	- - -	- - -	- - -	- - -	- - -	
546	L M	E Y S E Q T P V A L S K F G M A N K L I N Y Y R K K A N E Q D T L R P K L P V G E T H V L G V Q D	S. cerevisiae	- - -	- - -	- - -	- - -	- - -	
466	L V	E E S E E H P A V L S N A G M A S R I V N Y Y R K K N E Q D E S R P K L E V G E S H V L L D V Q D	S. pombe	- - -	- - -	- - -	- - -	- - -	
580	L A	E Y S E E N G P L M M Q V G M A T K I K N Y Y K R K P G K D P G A P D C K Y G E T V Y - - -	- - -	- - -	- - -	- - -	- - -	- - -	

A

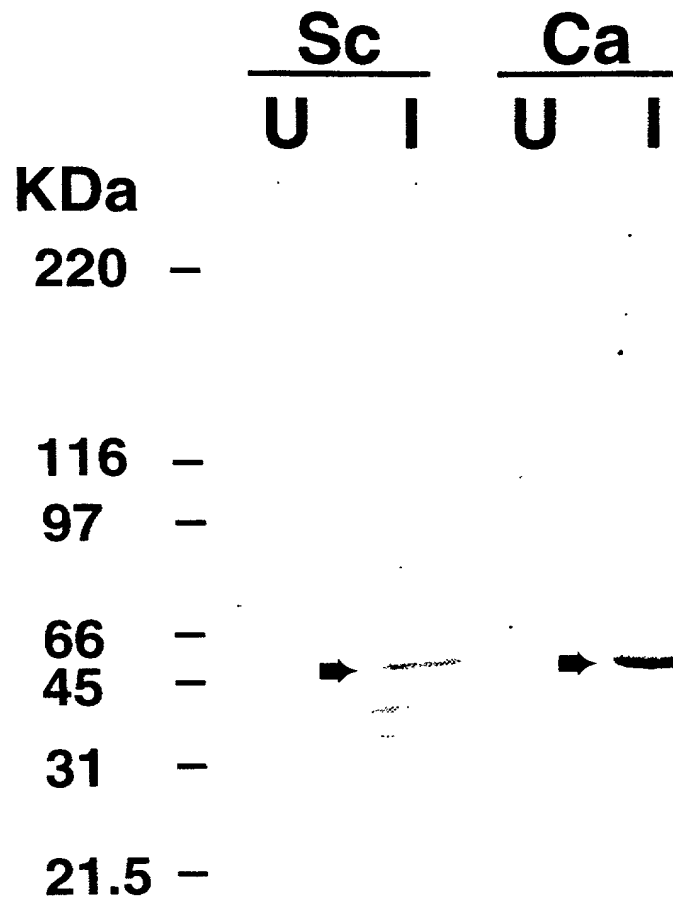
FIG. 8B

A		A	
581	R S P F W N F G E V A P G D F V P T T L Y N N M V R A P I F K H D N K P T D F L L V K S Q G A G S H Q	C. albicans	
596	K S P F W N F G E V E P G H I V P T T L Y N N M I R A P V F K H D I S G T D F L L T K S S G F G I S N S	S. cerevisiae	
516	R S P F W N F G S V E P G E I T P T T L Y N N M I R A P L L E K H E V P P T D F L I I R N S S - S Y G S S	S. pombe	
727	T S P F - - L G S L H P G Q L L Q A F E N N L F R A P I Y L H K M P E T D F L I I R T R - - - - - O	Human	
631	K F Y L R G I N F N F A V G N T F P V E - V P A P H S R K V T N I S K N R L K M V V F R V M - - N S	C. albicans	
646	R F Y L R N I N H L F T V G Q T F P V E E I P G P N S R K V T S M K A T R L K M I I Y R I L - - N H	S. cerevisiae	
565	K Y Y L K N I N H M F V S G Q T F P V T D V P G P H S R K V T T A S K N R L K M L V F R L I - - R R	S. pombe	
770	G Y I R E L V D I E V V G Q Q C P L F E V P G P N S K R A N I H I R D F L Q V F I Y R L F W K S K	Human	
678	L G V P R I S V K D V S K H F P E H S D M Q N R Q R L K E F M E Y Q R Q G E D Q G Y W K V R G L N D	C. albicans	
694	N H S K A I S I D P I A K H F P D Q D Y G Q N R Q K V K E F M K Y Q R D G P E K G L W R L K D - D E	S. cerevisiae	
613	S P N G G L F I R Q L S K H F S D Q N E M Q I R Q R L K E F M E Y K K K G D G P G Y W K L K S - N E	S. pombe	
820	D R P R R I R M E D I K K A E P S H S E S S I R K R L L C A D F K R T G M D S N W W L K S - D F	Human	
728	V I P G E E I R T M I T P E D S S L M D T M Q F G Q Q V L D D N M V L - F G E Q	C. albicans	
743	K L L D N E A V K S L I T P E Q I S Q V E S M S Q G L Q F Q E D N E A Y N F D S K - - L K S L	S. cerevisiae	
662	V V P D E A G I R S M V S P E T V C L L E S M Q V G V R Q L E D A - - - G Y G K T - - M D E	S. pombe	
869	R L P T E E E I R A M V S P E Q C C A Y Y S M I A A E Q R L K D A - - - G Y G E K S F F A P E	Human	



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FIG. 9



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FIG. 10A

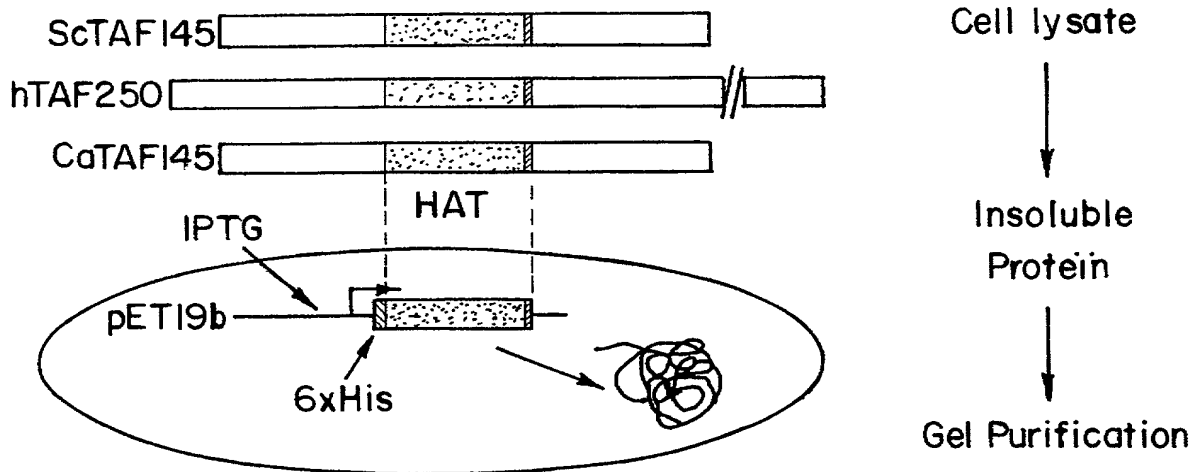
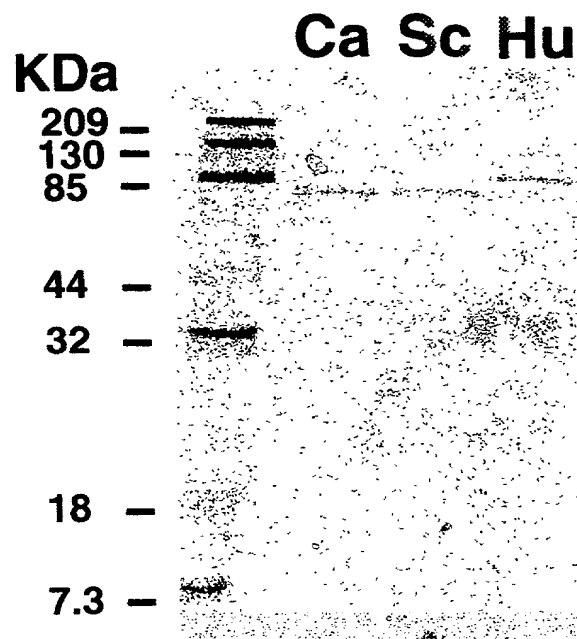


FIG. 10B



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FIG. 11

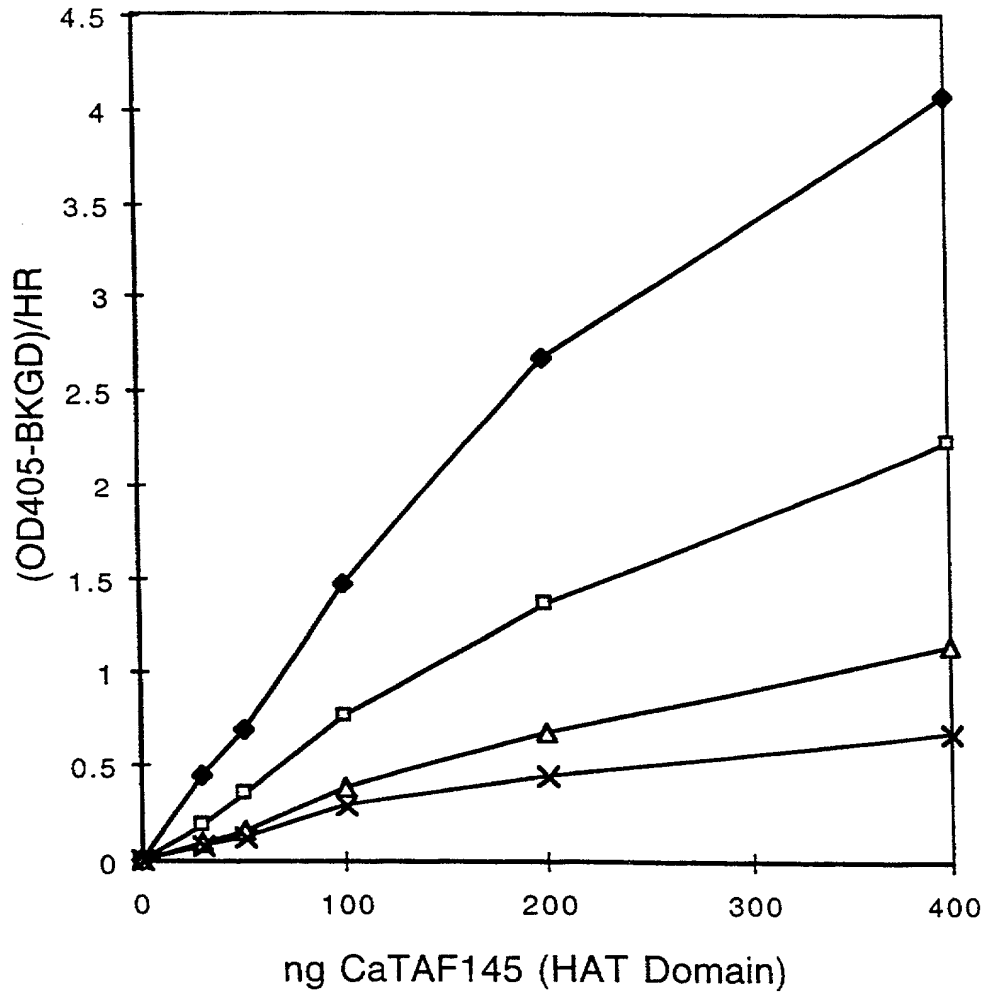
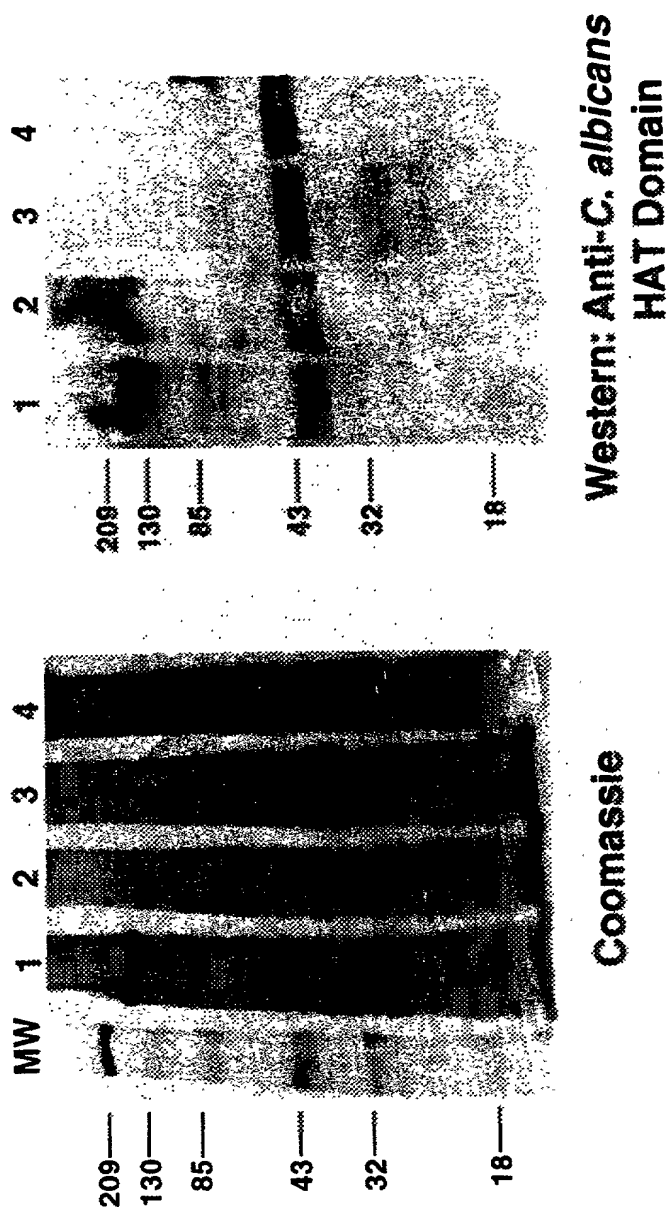
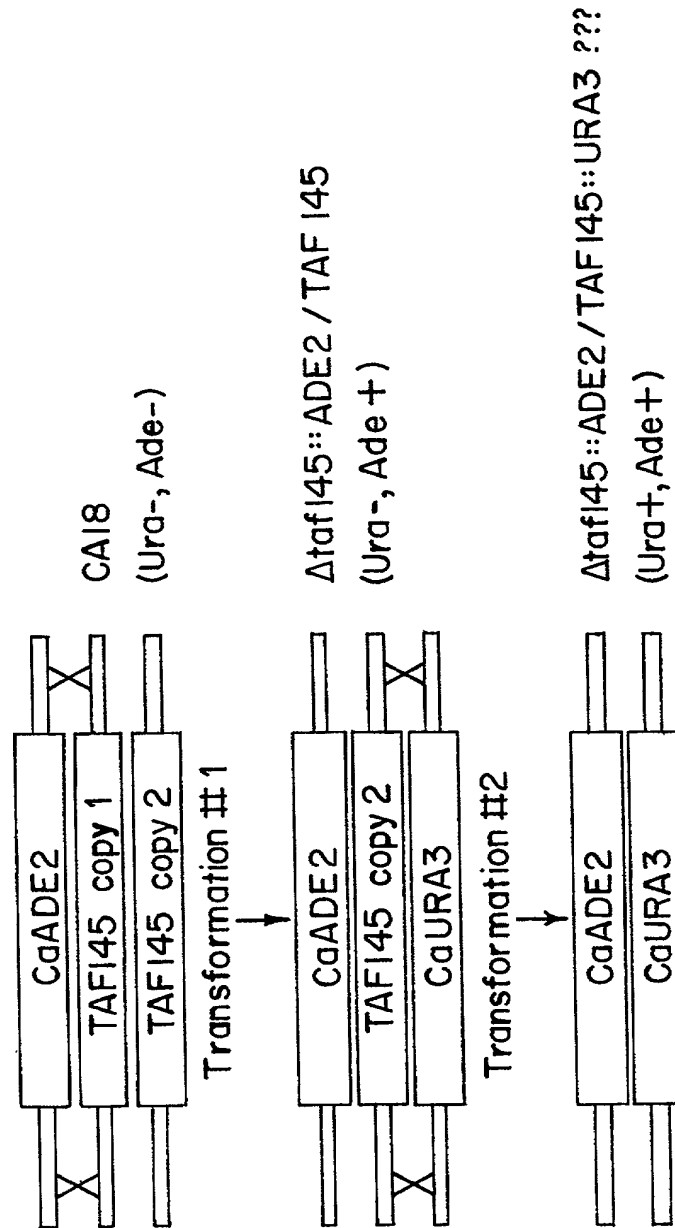


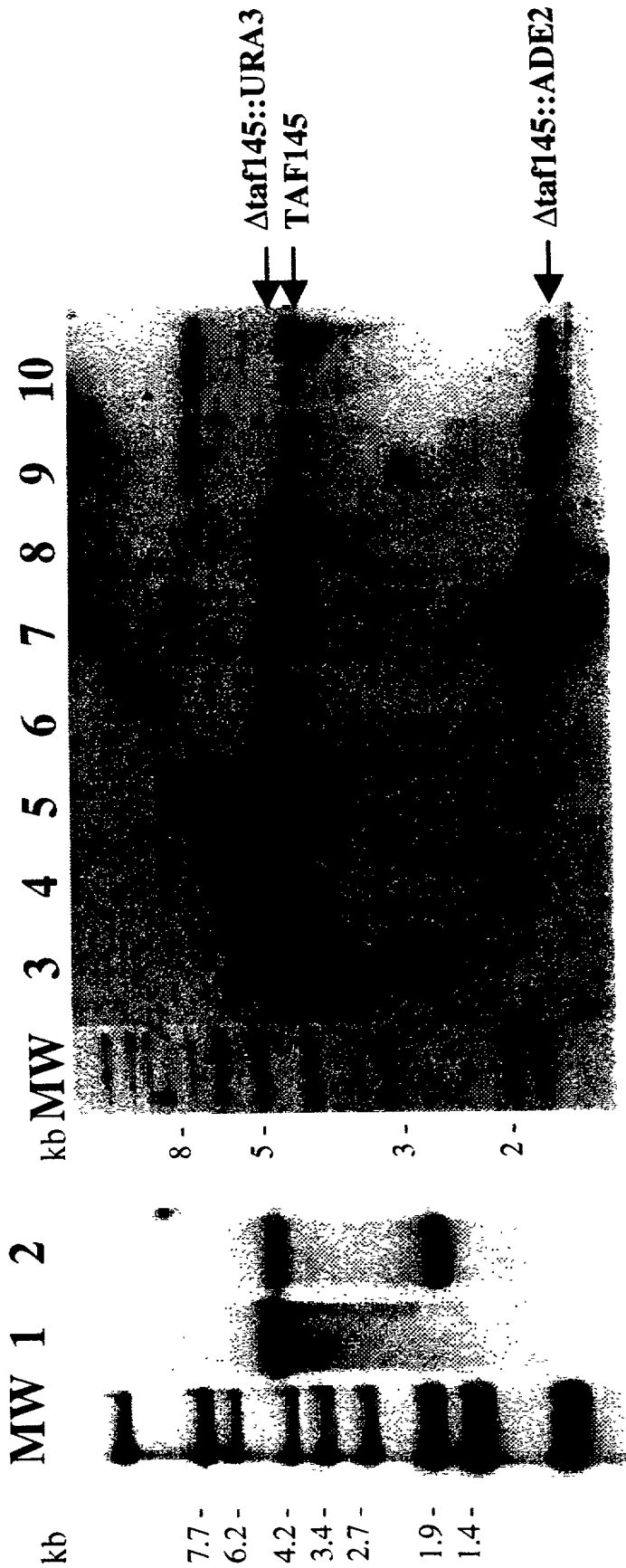
FIG. 12



1. Human TAF infected High-five cells
2. *C. albicans* TAF High-five infected cells
3. Mock-Infected High-five cells
4. Non-infected High-five cells

FIG. 13A





Lane

- 1 TAF145/TAF145 (CAI8)
- 2 Δtaf145::ADE2 / TAF145
- 3 TAF145 / Δtaf145::URA3
- 4-10 Δtaf145::ADE2 / TAF145, Ura+

FIG. 13B

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

(Includes Reference to PCT International Applications)

ATTORNEY DOCKET NUMBER

0342/1D516-US2

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed for and which a patent is sought on the invention entitled:

A NOVEL FUNGAL PROTEIN CRITICAL FOR EXPRESSION OF FUNGAL PROTEINS

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Serial No. _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/US99/02940

on 8 February 1999

and was amended under PCT Article 19

on _____ (if applicable).

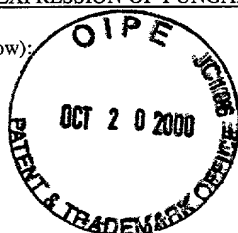
I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

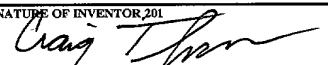
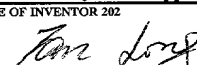

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119
US	60/074,100	9 February 1998	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
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			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO



Combined Declaration for Patent Application and Power of Attorney (Continued) (Includes Reference to PCT International Applications)				ATTY'S DOCKET NUMBER 0342/1D516-US2	
<p>I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:</p>					
PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:					
U.S. APPLICATIONS			STATUS (Check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED	
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. SERIAL NUMBER ASSIGNED (if any)			
<p>POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. <u>Morris Relson #15,108, Gordon D. Coplein #19,165, William F. Dudine, Jr. #20,569, Michael J. Sweedler #19,937, S. Peter Ludwig #25,351, Paul Fields #20,298, Joseph B. Lerch #26,936, Melvin C. Garner #26,272, Ethan Horwitz #27,646, Beverly B. Goodwin #28,417, Adda C. Gogoris #29,714, Martin E. Goldstein #20,869, Bert J. Lewen #19,407, Henry Sternberg #22,408, Peter C. Schechter #31,662, Robert Schaffer #31,194, David R. Francescani #25,159, Robert C. Sullivan, Jr. #30,499, and Joseph R. Robinson #33,448</u></p>					
Send Correspondence to: <div style="text-align: center;"> <u>DARBY & DARBY P.C.</u> <u>805 Third Avenue</u> <u>New York, New York 10022-7513</u> </div>			Direct Telephone Calls to: (name and telephone number) <div style="text-align: center;"> <u>(212) 527-7700</u> <u>Anne E. Zitron</u> </div>		
2	FULL NAME OF INVENTOR <u>Thompson</u>	FAMILY NAME <u>Thompson</u>	FIRST GIVEN NAME <u>Craig</u>	SECOND GIVEN NAME <u>M</u>	
0	RESIDENCE & CITIZENSHIP <u>Arlington</u>	CITY <u>Arlington</u>	STATE OR FOREIGN COUNTRY <u>Massachusetts MA</u>	COUNTRY OF CITIZENSHIP <u>U.S.A. ✓</u>	
1	POST OFFICE ADDRESS <u>15 College Avenue</u>	POST OFFICE ADDRESS <u>15 College Avenue</u>	CITY <u>Arlington</u>	STATE & ZIP CODE/COUNTRY <u>Massachusetts 02474</u>	
2	FULL NAME OF INVENTOR <u>Long</u>	FAMILY NAME <u>Long</u>	FIRST GIVEN NAME <u>Fan</u>	SECOND GIVEN NAME 	
0	RESIDENCE & CITIZENSHIP <u>Waltham</u>	CITY <u>Waltham</u>	STATE OR FOREIGN COUNTRY <u>Massachusetts MA</u>	COUNTRY OF CITIZENSHIP <u>China, PRC</u>	
2	POST OFFICE ADDRESS <u>51 Garden Circle, Apt. #10</u>	POST OFFICE ADDRESS <u>51 Garden Circle, Apt. #10</u>	CITY <u>Waltham</u>	STATE & ZIP CODE/COUNTRY <u>Massachusetts 02452</u>	
2	FULL NAME OF INVENTOR <u>Wobbe</u>	FAMILY NAME <u>Wobbe</u>	FIRST GIVEN NAME <u>Richard</u>	SECOND GIVEN NAME 	
0	RESIDENCE & CITIZENSHIP <u>Lexington</u>	CITY <u>Lexington</u>	STATE OR FOREIGN COUNTRY <u>Massachusetts MA</u>	COUNTRY OF CITIZENSHIP <u>U.S.A.</u>	
3	POST OFFICE ADDRESS <u>57 Spring Street</u>	POST OFFICE ADDRESS <u>57 Spring Street</u>	CITY <u>Lexington</u>	STATE & ZIP CODE/COUNTRY <u>Massachusetts 02173</u>	
<p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.</p>					
SIGNATURE OF INVENTOR 201 		SIGNATURE OF INVENTOR 202 		SIGNATURE OF INVENTOR 203 	
DATE <u>Aug. 24, 2000</u>		DATE <u>Oct 4, 2000</u>		DATE <u>Aug 25, 2000</u>	

SEQUENCE LISTING

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Thompson, M. Craig
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Wobbe, C. Richard

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COMPLEX CRITICAL FOR EXPRESSION OF FUNGAL PROTEINS

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 Arg Val Met Asn Ser Leu Gly Val Pro Arg Ile Ser Val Lys Asp Val
 675 680 685
 Ser Lys His Phe Pro Glu His Ser Asp Met Gln Asn Arg Gln Arg Leu
 690 695 700
 Lys Glu Phe Met Glu Tyr Gln Arg Gln Gly Glu Asp Gln Gly Tyr Trp
 705 710 715 720
 Lys Val Arg Gly Leu Asn Asp Val Ile Pro Gly Glu Glu Glu Ile Arg
 725 730 735
 Thr Met Ile Thr Pro Glu Asp Ser Ser Leu Met Asp Thr Met Gln Phe
 740 745 750

Gly Gln Gln Val Leu Asp Asp Asn Met Val Leu Phe Gly Glu Gln Ser
 755 760 765
 Arg Gln Glu Ser Ser Arg Ser Arg Lys Gly Asp Lys Arg Glu Asp Ser
 770 775 780
 Ile Ala Asp Asp Ala Glu Asn Gly Asp Asp Ile Asn Lys Asp Lys Glu
 785 790 795 800
 Lys Glu Val Glu Lys Glu Lys Glu Gln Glu Arg Glu Glu Glu Lys Gly
 805 810 815
 Lys Asp Lys Glu Lys Asp Lys Asp Lys Glu Lys Asp Lys Thr Glu Lys
 820 825 830
 Glu Lys Ser Lys Lys Ser Lys Glu Gln Asp Thr Glu Ile Asp Val Glu
 835 840 845
 Glu Glu Leu Ala Pro Trp Asn Leu Ser Arg Asn Phe Val Ile Ala Asn
 850 855 860
 Gln Thr Lys Thr Met Leu Gln Leu Asn Gly Glu Gly Asp Pro Thr Gly
 865 870 875 880
 Ile Gly Leu Gly Phe Ser Met Leu Arg Ala Thr Gln Lys Asn Pro Phe
 885 890 895
 Lys Pro Leu Phe Thr Pro Pro Pro Glu Asn Val Pro Lys Ser Asn Ala
 900 905 910
 Ala Ala His Asn Gln Lys Leu Tyr Glu Gln Glu Ile Lys Arg Ile Trp
 915 920 925
 Tyr Ser Gln Arg Ser Ser Leu Val Asp His Gly Glu Gly Thr Glu Ser
 930 935 940
 Lys Leu Gln Gln Ile Tyr Asn Glu Tyr Pro Pro Ala Asp His Glu Leu
 945 950 955 960
 Tyr Leu Lys Asn Lys Leu Glu Gln Asp Gln Gln Val Gln Gln Gln Gln
 965 970 975
 Gln Asp Pro Ser Leu Gln Ala Asp Gln Gln Gln Gln Gln Gln Gln
 980 985 990
 Gln Asn Arg Val Leu Arg Ile Thr Arg Arg Val Arg Asp Glu Asn Gly
 995 1000 1005
 Ile Val His Arg Lys Val Glu Phe Ile His Asp Pro Arg Leu Ile Arg
 1010 1015 1020
 Ala Tyr Val Lys Arg Lys Lys Gln Ile Glu Asp Glu Leu Leu Lys Asn
 1025 1030 1035 104
 Ala Asp Val Asp Glu Ile Leu Pro Thr Asn Asp Lys Glu Leu Asn Lys
 1045 1050 1055
 Ile Arg Arg Lys Ala Leu Glu Glu Lys Leu Ala Asn Leu Glu Lys Arg
 1060 1065 1070
 Ala Lys Gln Ser Arg Ala Lys Lys Pro Pro Lys Asp Leu Ile His Ala
 1075 1080 1085
 Ala Ala Ala Ala Gly Ala Thr Ile Ile Asp Ala Asn Thr Val Met Leu
 1090 1095 1100
 Pro Asp Gly Ser Tyr Val Ile Gly Gly Lys Gly Ile Gly Lys Gly Lys
 1105 1110 1115 112
 Ser Arg Thr Arg Arg Cys Lys Asn Cys Gly Ala Tyr Gly His Ile Arg
 1125 1130 1135
 Thr Asn Ala Lys Cys Pro Leu Tyr Lys Lys Met Val Leu Gly Ile Asp
 1140 1145 1150

Asp Asp Ser Ala Ala Val Val Gly
 1155 1160

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 <212> PRT
 <213> S. cerevisiae

<400> 3

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 Glu Ala Tyr Glu Ala Ile Phe Gly Gly Glu Phe Gly Ser Leu Glu Ile
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 Gly Ser Tyr Ile Gly Gly Asp Glu Gly Ala Asn Ser Lys Asp Tyr Thr
 35 40 45
 Glu His Leu Pro Asp Ala Val Asp Phe Glu Asp Glu Asp Glu Leu Ala
 50 55 60
 Asp Asp Asp Asp Asp Leu Pro Glu Glu Ser Asp Ala Asn Leu His Pro
 65 70 75 80
 Ala Met Met Thr Met Gly Ala Tyr Asp Asp Val Asn Glu Asn Gly Ala
 85 90 95
 Val Leu Gly Ile Asp Ser Asn Ser Leu Asn Met Gln Leu Pro Glu Ile
 100 105 110
 Asn Gly Asp Leu Ser Gln Gln Phe Ile Leu Glu Asp Asp Gly Gly Thr
 115 120 125
 Pro Ala Thr Ser Asn Ala Leu Phe Met Gly Met Asp Ala Asn Glu Ile
 130 135 140
 His Leu Ala Thr Glu Thr Gly Val Leu Asp Gly Ser Gly Ala Asn Glu
 145 150 155 160
 Ile Gly His Ser Gln Leu Ser Ile Gly Gly Val Asn Gly Asn Asp Met
 165 170 175
 Ser Ile Asn Gly Gly Phe Ile Met Glu Pro Asp Met Ser Asp Gly Lys
 180 185 190
 His Lys Lys Ala Thr Lys Leu Asp Leu Ile Asn His Glu Lys Tyr Leu
 195 200 205
 Leu Lys Lys Tyr Phe Pro Asp Phe Glu Lys Gly Lys Ile Leu Lys Trp
 210 215 220
 Asn Lys Leu Ile Tyr Arg Arg Ser Val Pro Tyr His Trp His Ser Glu
 225 230 235 240
 Ile Ser Arg Val Lys Lys Pro Phe Met Pro Leu Asn Leu Lys Phe Lys
 245 250 255
 Val Gln Gln Asp Asp Lys Arg Leu Phe Asn Ser Arg Thr Ile Ser Tyr
 260 265 270
 Val Ala Pro Ile Tyr Gln Gly Lys Asn Asn Leu Leu Gln Ser Asn Ser
 275 280 285
 Ser Ala Ser Arg Arg Gly Leu Ile His Val Ser Ile Asp Glu Leu Phe
 290 295 300
 Pro Ile Lys Glu Gln Gln Lys Lys Arg Lys Ile Ile His Asp Glu Lys
 305 310 315 320
 Thr Ile Ser Glu Asp Leu Leu Ile Ala Thr Asp Asp Trp Asp Gln Glu

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          325          330          335
Lys Ile Ile Asn Gln Gly Thr Ser Ser Thr Ala Thr Leu Ala Asp Ser
          340          345          350
Ser Met Thr Pro Asn Leu Lys Phe Ser Gly Gly Tyr Lys Leu Lys Ser
          355          360          365
Leu Ile Glu Asp Val Ala Glu Asp Trp Gln Trp Asp Glu Asp Met Ile
          370          375          380
Ile Asp Ala Lys Leu Lys Glu Ser Lys His Ala Glu Leu Asn Met Asn
385          390          395          400
Asp Glu Lys Leu Leu Leu Met Ile Glu Lys Thr Asn Asn Leu Ala Gln
          405          410          415
Gln Lys Gln Gln Leu Asp Ser Ser Asn Leu Ile Leu Pro Leu Asn Glu
          420          425          430
Thr Ile Leu Gln Gln Lys Phe Asn Leu Ser Asn Asp Asp Lys Tyr Gln
          435          440          445
Ile Leu Lys Lys Thr His Gln Thr Lys Val Arg Ser Thr Ile Ser Asn
450          455          460
Leu Asn Ile Gln His Ser Gln Pro Ala Ile Asn Leu Gln Ser Pro Phe
465          470          475          480
Tyr Lys Val Ala Val Pro Arg Tyr Gln Leu Arg His Phe His Arg Glu
          485          490          495
Asn Phe Gly Ser His Ile Arg Pro Gly Thr Lys Ile Val Phe Ser Lys
          500          505          510
Leu Lys Ala Arg Lys Arg Lys Arg Asp Lys Gly Lys Asp Val Lys Glu
          515          520          525
Ser Phe Ser Thr Ser Gln Asp Leu Thr Ile Gly Asp Thr Ala Pro Val
530          535          540
Tyr Leu Met Glu Tyr Ser Glu Gln Thr Pro Val Ala Leu Ser Lys Phe
545          550          555          560
Gly Met Ala Asn Lys Leu Ile Asn Tyr Tyr Arg Lys Ala Asn Glu Gln
          565          570          575
Asp Thr Leu Arg Pro Lys Leu Pro Val Gly Glu Thr His Val Leu Gly
          580          585          590
Val Gln Asp Lys Ser Pro Phe Trp Asn Phe Gly Phe Val Glu Pro Gly
          595          600          605
His Ile Val Pro Thr Leu Tyr Asn Asn Met Ile Arg Ala Pro Val Phe
610          615          620
Lys His Asp Ile Ser Gly Thr Asp Phe Leu Leu Thr Lys Ser Ser Gly
625          630          635          640
Phe Gly Ile Ser Asn Arg Phe Tyr Leu Arg Asn Ile Asn His Leu Phe
          645          650          655
Thr Val Gly Gln Thr Phe Pro Val Glu Glu Ile Pro Gly Pro Asn Ser
          660          665          670
Arg Lys Val Thr Ser Met Lys Ala Thr Arg Leu Lys Met Ile Ile Tyr
          675          680          685
Arg Ile Leu Asn His Asn His Ser Lys Ala Ile Ser Ile Asp Pro Ile
690          695          700
Ala Lys His Phe Pro Asp Gln Asp Tyr Gly Gln Asn Arg Gln Lys Val
705          710          715          720
Lys Glu Phe Met Lys Tyr Gln Arg Asp Gly Pro Glu Lys Gly Leu Trp

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725 730 735
 Arg Leu Lys Asp Asp Glu Lys Leu Leu Asp Asn Glu Ala Val Lys Ser
 740 745 750
 Leu Ile Thr Pro Glu Gln Ile Ser Gln Val Glu Ser Met Ser Gln Gly
 755 760 765
 Leu Gln Phe Gln Glu Asp Asn Glu Ala Tyr Asn Phe Asp Ser Lys Leu
 770 775 780
 Lys Ser Leu Glu Glu Asn Leu Leu Pro Trp Asn Ile Thr Lys Asn Phe
 785 790 795 800
 Ile Asn Ser Thr Gln Met Arg Ala Met Ile Gln Ile His Gly Val Gly
 805 810 815
 Asp Pro Thr Gly Cys Gly Glu Gly Phe Ser Phe Leu Lys Thr Ser Met
 820 825 830
 Lys Gly Gly Phe Val Lys Ser Gly Ser Pro Ser Ser Asn Asn Asn Ser
 835 840 845
 Ser Asn Lys Lys Gly Thr Asn Thr His Ser Tyr Asn Val Ala Gln Gln
 850 855 860
 Gln Lys Ala Tyr Asp Glu Glu Ile Ala Lys Thr Trp Tyr Thr His Thr
 865 870 875 880
 Lys Ser Leu Ser Ile Ser Asn Pro Phe Glu Glu Met Thr Asn Pro Asp
 885 890 895
 Glu Ile Asn Gln Thr Asn Lys His Val Lys Thr Asp Arg Asp Asp Lys
 900 905 910
 Lys Ile Leu Lys Ile Val Arg Lys Lys Arg Asp Glu Asn Gly Ile Ile
 915 920 925
 Gln Arg Gln Thr Ile Phe Ile Arg Asp Pro Arg Val Ile Gln Gly Tyr
 930 935 940
 Ile Lys Ile Lys Glu Gln Asp Lys Glu Asp Val Asn Lys Leu Leu Glu
 945 950 955 960
 Glu Asp Thr Ser Lys Ile Asn Asn Leu Glu Glu Leu Glu Lys Gln Lys
 965 970 975
 Lys Leu Leu Gln Leu Glu Leu Ala Asn Leu Glu Lys Ser Gln Gln Arg
 980 985 990
 Arg Ala Ala Arg Gln Asn Ser Lys Arg Asn Gly Gly Ala Thr Arg Thr
 995 1000 1005
 Glu Asn Ser Val Asp Asn Gly Ser Asp Leu Ala Gly Val Thr Asp Gly
 1010 1015 1020
 Lys Ala Ala Arg Asn Lys Gly Lys Asn Thr Thr Arg Arg Cys Ala Thr
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 Cys Gly Gln Ile Gly His Ile Arg Thr Asn Lys Ser Cys Pro Met Tyr
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 Ser Ser Lys Asp Asn Pro Ala Ser Pro Lys
 1060 1065

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<211> 979

<212> PRT

<213> S. pombe

<400> 4

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 Tyr Asn Asp Gly Asn Asp Leu Thr Asp Leu Phe Lys Gln Asn Gly Thr
 20 25 30
 Asp Met Ser Val Ile Asn Ser Leu Leu Gly Asp Thr Asn Asn Pro Gly
 35 40 45
 Met Asn Glu Ser Pro Lys Ile Leu Asp Ser Ser Phe Glu Asn Ser Asn
 50 55 60
 Pro Gln Asp Gly Pro Asn Tyr Glu Asp Phe Asp Phe Met Gly Ser Ile
 65 70 75 80
 His Lys Glu Phe Gly Asn Asn Ile Asn Glu Met Asp Asp Met Glu Asp
 85 90 95
 Val Ser Asp Asp Asn Leu Pro Glu Glu Glu Gln Ala Val Asn Tyr Thr
 100 105 110
 Gly Asp Lys Asp Asp Glu Asp Phe Gly Lys Leu Leu Ala Lys Glu Met
 115 120 125
 Gly Glu Glu Ala Ala Gly Gln Val Leu Ser Gly Val Gly Phe Ser Ile
 130 135 140
 Pro Ser Gly Leu Val Pro Pro Ser Glu Pro Ser Lys Thr Val Ser Ser
 145 150 155 160
 Thr Thr Glu Glu Leu Gln Asn Glu Ala Gln Ile Arg Glu Ser Ile Val
 165 170 175
 Lys Thr Phe Phe Pro Thr Phe Glu Arg Gly Val Leu Leu Asn Phe Ser
 180 185 190
 Glu Leu Phe Lys Pro Lys Pro Val Lys Leu Ala Pro Pro Lys Lys Lys
 195 200 205
 Thr Pro Lys Val Cys Val Pro Gly Arg Leu Thr Leu Glu Val Asp Thr
 210 215 220
 Asp Tyr Ala Ile Ile Phe Asn Ser Lys Lys Ser Leu Pro Leu Lys Arg
 225 230 235 240
 Asn Val Val Ser Pro Ile Ser Thr His Thr Lys Lys Arg Arg Arg Thr
 245 250 255
 Ala Asn Thr Ser Gln Arg Asn Asp Gly Leu Asp Leu Asn Thr Val Phe
 260 265 270
 Thr Thr Asn Asp Trp Glu Lys Asn Ile Ile Tyr Asp Glu Ser Asp Val
 275 280 285
 Asn Lys Thr Asn Gln Ser Ser Phe Phe Ile Asp Lys Ser Leu Val Asp
 290 295 300
 Ile Asp Phe Ala Phe Asp Glu Asn Ile Phe Asp Gly Asp Thr Gly Thr
 305 310 315 320
 Ser Lys Val Val Leu Asn Leu Asn Asp Pro Lys Leu Leu Leu Gln Pro
 325 330 335
 Gln Leu Pro Lys Lys Glu Asp Ser Gln Arg Ser Phe Ala Asp Thr His
 340 345 350
 Gln Arg Asn Ser Leu Ala Trp Lys Phe Asn Ile Ser Asn Asp Pro Ala
 355 360 365
 Tyr Glu Met Leu Lys Gln Asn His Gln Ser Lys Val Arg Asn Thr Leu
 370 375 380
 Ser Gln Leu Ala Ile Glu His Ala Ala Phe Ala Glu Lys Leu Thr Phe
 385 390 395 400

Pro Tyr Tyr Lys Thr Arg Leu Ser Lys Arg Ala Val Arg Ser Tyr His
 405 410 415
 Arg Pro Thr Met Ser Phe Lys Pro Asn Ala Ala Ile Val Phe Ser Pro
 420 425 430
 Leu Ile Val Arg Lys Arg Ser Lys Asp Lys His Lys Ser Glu Arg Glu
 435 440 445
 Leu Ile Pro Thr Thr Lys Glu Ile Thr Met Gly Asp Thr Thr His Ala
 450 455 460
 Ile Leu Val Glu Phe Ser Glu Glu His Pro Ala Val Leu Ser Asn Ala
 465 470 475 480
 Gly Met Ala Ser Arg Ile Val Asn Tyr Tyr Arg Lys Lys Asn Glu Gln
 485 490 495
 Asp Glu Ser Arg Pro Lys Leu Glu Val Gly Glu Ser His Val Leu Asp
 500 505 510
 Val Gln Asp Arg Ser Pro Phe Trp Asn Phe Gly Ser Val Glu Pro Gly
 515 520 525
 Glu Ile Thr Pro Thr Leu Tyr Asn Lys Met Ile Arg Ala Pro Leu Phe
 530 535 540
 Lys His Glu Val Pro Pro Thr Asp Phe Ile Leu Ile Arg Asn Ser Ser
 545 550 555 560
 Ser Tyr Gly Ser Lys Tyr Tyr Leu Lys Asn Ile Asn His Met Phe Val
 565 570 575
 Ser Gly Gln Thr Phe Pro Val Thr Asp Val Pro Gly Pro His Ser Arg
 580 585 590
 Lys Val Thr Thr Ala Ser Lys Asn Arg Leu Lys Met Leu Val Phe Arg
 595 600 605
 Leu Ile Arg Arg Ser Pro Asn Gly Gly Leu Phe Ile Arg Gln Leu Ser
 610 615 620
 Lys His Phe Ser Asp Gln Asn Glu Met Gln Ile Arg Gln Arg Leu Lys
 625 630 635 640
 Glu Phe Met Glu Tyr Lys Lys Lys Gly Asp Gly Pro Gly Tyr Trp Lys
 645 650 655
 Leu Lys Ser Asn Glu Val Val Pro Asp Glu Ala Gly Thr Arg Ser Met
 660 665 670
 Val Ser Pro Glu Thr Val Cys Leu Leu Glu Ser Met Gln Val Gly Val
 675 680 685
 Arg Gln Leu Glu Asp Ala Gly Tyr Gly Lys Thr Met Asp Glu Ile Asn
 690 695 700
 Asp Asp Glu Asp Glu Glu Gln Pro Ala Glu Gln Leu Leu Ala Pro Trp
 705 710 715 720
 Ile Thr Thr Arg Asn Phe Ile Asn Ala Thr Gln Gly Lys Ala Met Leu
 725 730 735
 Thr Leu Phe Gly Glu Gly Asp Pro Thr Gly Ile Gly Glu Gly Tyr Ser
 740 745 750
 Phe Ile Arg Thr Ser Met Lys Gly Gly Phe Lys Pro Ala Gly Glu Thr
 755 760 765
 Ala Asp Asp Lys Pro Glu Pro Gln Thr Lys Asn Ala His Ala Tyr Asn
 770 775 780
 Val Ala Lys Gln Gln Arg Ala Tyr Glu Glu Glu Ile Asn Arg Ile Trp
 785 790 795 800

Genotype		Phenotype	
Genotype	Phenotype	Genotype	Phenotype
100%	100%	100%	100%
90%	90%	90%	90%
80%	80%	80%	80%
70%	70%	70%	70%
60%	60%	60%	60%
50%	50%	50%	50%
40%	40%	40%	40%
30%	30%	30%	30%
20%	20%	20%	20%
10%	10%	10%	10%
0%	0%	0%	0%

545 550 555 560
 Asn Lys Ser Thr Gly Pro Pro Pro Lys Ile Lys Ile Pro His Pro His
 565 570 575
 Val Lys Lys Ser Lys Ile Leu Leu Gly Lys Ala Gly Val Ile Asn Val
 580 585 590
 Leu Ala Glu Asp Thr Pro Pro Pro Pro Pro Lys Ser Pro Asp Arg Asp
 595 600 605
 Pro Phe Asn Ile Ser Asn Asp Thr Tyr Tyr Thr Pro Lys Thr Glu Pro
 610 615 620
 Thr Leu Arg Leu Lys Val Gly Gly Asn Leu Ile Gln His Ser Thr Pro
 625 630 635 640
 Val Val Glu Leu Arg Ala Pro Phe Val Pro Thr His Met Gly Pro Met
 645 650 655
 Asn Val Arg Ala Phe His Arg Pro Pro Leu Lys Lys Tyr Ser His Gly
 660 665 670
 Pro Met Ala Gln Ser Ile Pro His Pro Val Phe Pro Leu Leu Lys Thr
 675 680 685
 Ile Ala Lys Lys Ala Lys Gln Arg Glu Val Glu Arg Ile Ala Ser Gly
 690 695 700
 Gly Gly Asp Val Phe Phe Met Arg Asn Pro Glu Asp Leu Ser Gly Arg
 705 710 715 720
 Asp Gly Asp Ile Val Leu Ala Glu Phe Cys Glu Glu His Pro Pro Leu
 725 730 735
 Ile Asn Gln Val Gly Met Cys Ser Lys Ile Lys Asn Tyr Tyr Lys Arg
 740 745 750
 Lys Ala Glu Lys Asp Ser Gly Pro Gln Asp Tyr Val Tyr Gly Glu Val
 755 760 765
 Ala Phe Ala His Thr Ser Pro Phe Leu Gly Ile Leu His Pro Gly Gln
 770 775 780
 Cys Ile Gln Ala Ile Glu Asn Asn Met Tyr Arg Ala Pro Ile Tyr Pro
 785 790 795 800
 His Lys Met Ala His Asn Asp Phe Leu Val Ile Arg Thr Arg Asn Asn
 805 810 815
 Tyr Trp Ile Arg Ser Val Asn Ser Ile Tyr Thr Val Gly Gln Glu Cys
 820 825 830
 Pro Leu Tyr Glu Val Pro Gly Pro Asn Ser Lys Arg Ala Asn Asn Phe
 835 840 845
 Thr Arg Asp Phe Leu Gln Val Phe Ile Tyr Arg Leu Phe Trp Lys Ser
 850 855 860
 Arg Asp Asn Pro Arg Arg Ile Arg Met Asp Asp Ile Lys Gln Ala Phe
 865 870 875 880
 Pro Ala His Ser Glu Ser Ser Ile Arg Lys Arg Leu Lys Gln Cys Ala
 885 890 895
 Asp Phe Lys Arg Thr Gly Met Asp Ser Asn Trp Trp Val Ile Lys Pro
 900 905 910
 Glu Phe Arg Leu Pro Ser Glu Glu Ile Arg Ala Met Val Ser Pro
 915 920 925
 Glu Gln Cys Cys Ala Tyr Phe Ser Met Ile Ala Ala Glu Gln Arg Leu
 930 935 940
 Lys Asp Ala Gly Tyr Gly Glu Lys Phe Leu Phe Ala Pro Gln Glu Asp

945 950 955 960
 Asp Asp Glu Glu Ala Gln Leu Lys Leu Asp Asp Glu Val Lys Val Ala
 965 970 975
 Pro Trp Asn Thr Thr Arg Ala Tyr Ile Gln Ala Met Arg Gly Lys Cys
 980 985 990
 Leu Leu Gln Leu Ser Gly Pro Ala Asp Pro Thr Gly Cys Gly Glu Gly
 995 1000 1005
 Phe Ser Tyr Val Arg Val Pro Asn Lys Pro Thr Gln Thr Lys Glu Glu
 1010 1015 1020
 Gln Glu Ser Gln Pro Lys Arg Ser Val Thr Gly Thr Asp Ala Asp Leu
 1025 1030 1035 104
 Arg Arg Leu Pro Leu Gln Arg Ala Lys Glu Leu Leu Arg Gln Phe Lys
 1045 1050 1055
 Val Pro Glu Glu Glu Ile Lys Lys Leu Ser Arg Trp Glu Val Ile Asp
 1060 1065 1070
 Val Val Arg Thr Leu Ser Thr Glu Lys Ala Lys Ala Gly Glu Glu Gly
 1075 1080 1085
 Met Asp Lys Phe Ser Arg Gly Asn Arg Phe Ser Ile Ala Glu His Gln
 1090 1095 1100
 Glu Arg Tyr Lys Glu Glu Cys Gln Arg Ile Phe Asp Leu Gln Asn Arg
 1105 1110 1115 112
 Val Leu Ala Ser Ser Glu Val Leu Ser Thr Asp Glu Ala Glu Ser Ser
 1125 1130 1135
 Ala Ser Glu Glu Ser Asp Leu Glu Glu Leu Gly Lys Asn Leu Glu Asn
 1140 1145 1150
 Met Leu Ser Asn Lys Lys Thr Ser Thr Gln Leu Ser Arg Glu Arg Glu
 1155 1160 1165
 Glu Leu Glu Arg Gln Glu Leu Leu Arg Gln Leu Asp Glu Glu His Gly
 1170 1175 1180
 Gly Pro Ser Gly Ser Gly Gly Ala Lys Gly Ala Lys Gly Lys Asp Asp
 1185 1190 1195 120
 Pro Gly Gln Gln Met Leu Ala Thr Asn Asn Gln Gly Arg Ile Leu Arg
 1205 1210 1215
 Ile Thr Arg Thr Phe Arg Gly Asn Asp Gly Lys Glu Tyr Thr Arg Val
 1220 1225 1230
 Glu Thr Val Arg Arg Gln Pro Val Ile Asp Ala Tyr Ile Lys Ile Arg
 1235 1240 1245
 Thr Thr Lys Asp Glu Gln Phe Ile Lys Gln Phe Ala Thr Leu Asp Glu
 1250 1255 1260
 Gln Gln Lys Glu Glu Met Lys Arg Glu Lys Arg Arg Ile Gln Glu Gln
 1265 1270 1275 128
 Leu Arg Arg Ile Lys Arg Asn Gln Glu Arg Glu Arg Leu Ala Gln Leu
 1285 1290 1295
 Ala Gln Asn Gln Lys Leu Gln Pro Gly Gly Met Pro Thr Ser Leu Gly
 1300 1305 1310
 Asp Pro Lys Ser Ser Gly Gly His Ser His Lys Glu Arg Asp Ser Gly
 1315 1320 1325
 Tyr Lys Glu Val Ser Pro Ser Arg Lys Lys Phe Lys Leu Lys Pro Asp
 1330 1335 1340
 Leu Lys Leu Lys Cys Gly Ala Cys Gly Gln Val Gly His Met Arg Thr

[illegible]

1745 1750 1755 176
 Ser Ile His Arg Ser Met Gly Ala Glu Ala Gly Ser Ser His Thr Ala
 1765 1770 1775
 Pro Ala Val Arg Lys Pro Ala Pro Pro Gly Pro Gly Glu Val Lys Arg
 1780 1785 1790
 Gly Arg Gly Arg Pro Arg Lys Gln Arg Asp Pro Val Glu Glu Asp Leu
 1795 1800 1805
 Gln Cys Ser Thr Asp Asp Glu Asp Asp Asp Glu Glu Glu Asp Phe Gln
 1810 1815 1820
 Glu Val Ser Glu Asp Glu Asn Asn Ala Ala Ser Ile Leu Asp Gln Gly
 1825 1830 1835 184
 Glu Arg Ile Asn Ala Pro Ala Asp Ala Met Asp Gly Met Phe Asp Pro
 1845 1850 1855
 Lys Asn Ile Lys Thr Glu Ile Asp Leu Glu Ala His Gln Met Ala Asp
 1860 1865 1870
 Glu Ser Met Asp Val Asp Pro Asn Tyr Asp Pro Ser Asp Phe Leu Ala
 1875 1880 1885
 Met His Lys Gln Arg Gln Ser Leu Gly Glu Pro Ser Ser Leu Gln Gly
 1890 1895 1900
 Ala Phe Thr Asn Phe Leu Ser His Glu Gln Asp Asp Asn Gly Pro Tyr
 1905 1910 1915 192
 Asn Pro Ala Glu Ala Ser Thr Ser Ala Ala Ser Gly Ala Asp Leu Gly
 1925 1930 1935
 Met Asp Ala Ser Met Ala Met Gln Met Ala Pro Glu Met Pro Val Asn
 1940 1945 1950
 Thr Met Asn Asn Gly Met Gly Ile Asp Asp Asp Leu Asp Ile Ser Glu
 1955 1960 1965
 Ser Asp Glu Glu Asp Asp Gly Ser Arg Val Arg Ile Lys Lys Glu Val
 1970 1975 1980
 Phe Asp Asp Gly Asp Tyr Ala Leu Gln His Gln Gln Met Gly Gln Ala
 1985 1990 1995 200
 Ala Ser Gln Ser Gln Ile Tyr Met Val Asp Ser Ser Asn Glu Pro Thr
 2005 2010 2015
 Thr Leu Asp Tyr Gln Gln Pro Pro Gln Leu Asp Phe Gln Gln Val Gln
 2020 2025 2030
 Glu Met Glu Gln Leu Gln His Gln Val Met Pro Pro Met Gln Ser Glu
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 Gln Leu Gln Gln Gln Gln Thr Pro Gln Gly Asp Asn Asp Tyr Ala Trp
 2050 2055 2060
 Thr Phe
 2065

<210> 6
 <211> 1872
 <212> PRT
 <213> Human

<400> 6

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Ala Ala Ala Ile Met Ser Asp Thr Asp Ser Asp Glu Asp Ser Ala Gly
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 35 40 45
 Ala Gly Gln Leu Glu Gly Glu Ser Val Leu Asp Asp Glu Cys Lys Lys
 50 55 60
 His Leu Ala Gly Leu Gly Ala Leu Gly Leu Gly Ser Leu Ile Thr Glu
 65 70 75 80
 Leu Thr Ala Asn Glu Glu Leu Thr Gly Thr Asp Gly Ala Leu Val Asn
 85 90 95
 Asp Glu Gly Trp Val Arg Ser Thr Glu Asp Ala Val Asp Tyr Ser Asp
 100 105 110
 Ile Asn Glu Val Ala Glu Asp Glu Ser Arg Arg Tyr Gln Gln Thr Met
 115 120 125
 Gly Ser Leu Gln Pro Leu Cys His Ser Asp Tyr Asp Glu Asp Asp Tyr
 130 135 140
 Asp Ala Asp Cys Glu Asp Ile Asp Cys Lys Leu Met Pro Pro Pro Pro
 145 150 155 160
 Pro Pro Pro Gly Pro Met Lys Lys Asp Lys Asp Gln Asp Ser Ile Thr
 165 170 175
 Gly Glu Lys Val Asp Phe Ser Ser Ser Ser Asp Ser Glu Ser Glu Met
 180 185 190
 Gly Pro Gln Glu Ala Thr Gln Ala Glu Ser Glu Asp Gly Lys Leu Thr
 195 200 205
 Leu Pro Leu Ala Gly Ile Met Gln His Asp Ala Thr Lys Leu Leu Pro
 210 215 220
 Ser Val Thr Glu Leu Phe Pro Glu Phe Arg Pro Gly Lys Val Leu Arg
 225 230 235 240
 Phe Leu Arg Leu Phe Gly Pro Gly Lys Asn Val Pro Ser Val Trp Arg
 245 250 255
 Ser Ala Arg Arg Lys Arg Lys Lys Lys His Arg Glu Leu Ile Gln Glu
 260 265 270
 Glu Gln Ile Gln Glu Val Glu Cys Ser Val Glu Ser Glu Val Ser Gln
 275 280 285
 Lys Ser Leu Trp Asn Tyr Asp Tyr Ala Pro Pro Pro Pro Pro Glu Gln
 290 295 300
 Cys Leu Ser Asp Asp Glu Ile Thr Met Met Ala Pro Val Glu Ser Lys
 305 310 315 320
 Phe Ser Gln Ser Thr Gly Asp Ile Asp Lys Val Thr Asp Thr Lys Pro
 325 330 335
 Arg Val Ala Glu Trp Arg Tyr Gly Pro Ala Arg Leu Trp Tyr Asp Met
 340 345 350
 Leu Gly Val Pro Glu Asp Gly Ser Gly Phe Asp Tyr Gly Phe Lys Leu
 355 360 365
 Arg Lys Thr Glu His Glu Pro Val Ile Lys Ser Arg Met Ile Glu Glu
 370 375 380
 Phe Arg Lys Leu Glu Glu Asn Asn Gly Thr Asp Leu Leu Ala Asp Glu
 385 390 395 400
 Asn Phe Leu Met Val Thr Gln Leu His Trp Glu Asp Asp Ile Ile Trp
 405 410 415

Asp Gly Glu Asp Val Lys His Lys Gly Thr Lys Pro Gln Arg Ala Ser
 420 425 430
 Leu Ala Gly Trp Leu Pro Ser Ser Met Thr Arg Asn Ala Met Ala Tyr
 435 440 445
 Asn Val Gln Gln Gly Phe Ala Ala Thr Leu Asp Asp Asp Lys Pro Trp
 450 455 460
 Tyr Ser Ile Phe Pro Ile Asp Asn Glu Asp Leu Val Tyr Gly Arg Trp
 465 470 475 480
 Glu Asp Asn Ile Ile Trp Asp Ala Gln Ala Met Pro Arg Leu Leu Glu
 485 490 495
 Pro Pro Val Leu Thr Leu Asp Pro Asn Asp Glu Asn Leu Ile Leu Glu
 500 505 510
 Ile Pro Asp Glu Lys Glu Glu Ala Thr Ser Asn Ser Pro Ser Lys Glu
 515 520 525
 Ser Lys Lys Glu Ser Ser Leu Lys Lys Ser Arg Ile Leu Leu Gly Lys
 530 535 540
 Thr Gly Val Ile Lys Glu Glu Pro Gln Gln Asn Met Ser Gln Pro Glu
 545 550 555 560
 Val Lys Asp Pro Trp Asn Leu Ser Asn Asp Glu Tyr Tyr Tyr Pro Lys
 565 570 575
 Gln Gln Gly Leu Arg Gly Thr Phe Gly Gly Asn Ile Ile Gln His Ser
 580 585 590
 Ile Pro Ala Val Glu Leu Arg Gln Pro Phe Phe Pro Thr His Met Gly
 595 600 605
 Pro Ile Lys Leu Arg Gln Phe His Arg Pro Pro Leu Lys Lys Tyr Ser
 610 615 620
 Phe Gly Ala Leu Ser Gln Pro Gly Pro His Ser Val Gln Pro Leu Leu
 625 630 635 640
 Lys His Ile Lys Lys Lys Ala Lys Met Arg Glu Gln Glu Arg Gln Ala
 645 650 655
 Ser Gly Gly Gly Glu Met Phe Phe Met Arg Thr Pro Gln Asp Leu Thr
 660 665 670
 Gly Lys Asp Gly Asp Leu Ile Leu Ala Glu Tyr Ser Glu Glu Asn Gly
 675 680 685
 Pro Leu Met Met Gln Val Gly Met Ala Thr Lys Ile Lys Asn Tyr Tyr
 690 695 700
 Lys Arg Lys Pro Gly Lys Asp Pro Gly Ala Pro Asp Cys Lys Tyr Gly
 705 710 715 720
 Glu Thr Val Tyr Cys His Thr Ser Pro Phe Leu Gly Ser Leu His Pro
 725 730 735
 Gly Gln Leu Leu Gln Ala Phe Glu Asn Leu Phe Arg Ala Pro Ile
 740 745 750
 Tyr Leu His Lys Met Pro Glu Thr Asp Phe Leu Ile Ile Arg Thr Arg
 755 760 765
 Gln Gly Tyr Tyr Ile Arg Glu Leu Val Asp Ile Phe Val Val Gly Gln
 770 775 780
 Gln Cys Pro Leu Phe Glu Val Pro Gly Pro Asn Ser Lys Arg Ala Asn
 785 790 795 800
 Thr His Ile Arg Asp Phe Leu Gln Val Phe Ile Tyr Arg Leu Phe Trp
 805 810 815

Lys Ser Lys Asp Arg Pro Arg Arg Ile Arg Met Glu Asp Ile Lys Lys
 820 825 830
 Ala Phe Pro Ser His Ser Glu Ser Ser Ile Arg Lys Arg Leu Lys Leu
 835 840 845
 Cys Ala Asp Phe Lys Arg Thr Gly Met Asp Ser Asn Trp Trp Val Leu
 850 855 860
 Lys Ser Asp Phe Arg Leu Pro Thr Glu Glu Glu Ile Arg Ala Met Val
 865 870 875 880
 Ser Pro Glu Gln Cys Cys Ala Tyr Tyr Ser Met Ile Ala Ala Glu Gln
 885 890 895
 Arg Leu Lys Asp Ala Gly Tyr Gly Glu Lys Ser Phe Phe Ala Pro Glu
 900 905 910
 Glu Glu Asn Glu Glu Asp Phe Gln Met Lys Ile Asp Asp Glu Val Arg
 915 920 925
 Thr Ala Pro Trp Asn Thr Thr Arg Ala Phe Ile Ala Ala Met Lys Gly
 930 935 940
 Lys Cys Leu Leu Glu Val Thr Gly Val Ala Asp Pro Thr Gly Cys Gly
 945 950 955 960
 Glu Gly Phe Ser Tyr Val Lys Ile Pro Asn Lys Pro Thr Gln Gln Lys
 965 970 975
 Asp Asp Lys Glu Pro Gln Pro Val Lys Lys Thr Val Thr Gly Thr Asp
 980 985 990
 Ala Asp Leu Arg Arg Leu Ser Leu Lys Asn Ala Lys Gln Leu Leu Arg
 995 1000 1005
 Lys Phe Gly Val Pro Glu Glu Glu Ile Lys Lys Leu Ser Arg Trp Glu
 1010 1015 1020
 Val Ile Asp Val Val Arg Thr Met Ser Thr Glu Gln Ala Arg Ser Gly
 1025 1030 1035 104
 Glu Gly Pro Met Ser Lys Phe Ala Arg Gly Ser Arg Phe Ser Val Ala
 1045 1050 1055
 Glu His Gln Glu Arg Tyr Lys Glu Glu Cys Gln Arg Ile Phe Asp Leu
 1060 1065 1070
 Gln Asn Lys Val Leu Ser Ser Thr Glu Val Leu Ser Thr Asp Thr Asp
 1075 1080 1085
 Ser Ser Ser Ala Glu Asp Ser Asp Phe Glu Glu Met Gly Lys Asn Ile
 1090 1095 1100
 Glu Asn Met Leu Gln Asn Lys Lys Thr Ser Ser Gln Leu Ser Arg Glu
 1105 1110 1115 112
 Arg Glu Glu Gln Glu Arg Lys Glu Leu Gln Arg Met Leu Leu Ala Ala
 1125 1130 1135
 Gly Ser Ala Ala Ser Gly Asn Asn His Arg Asp Asp Asp Thr Ala Ser
 1140 1145 1150
 Val Thr Ser Leu Asn Ser Ser Ala Thr Gly Arg Cys Leu Lys Ile Tyr
 1155 1160 1165
 Arg Thr Phe Arg Asp Glu Glu Gly Lys Glu Tyr Val Arg Cys Glu Thr
 1170 1175 1180
 Val Arg Lys Pro Ala Val Ile Asp Ala Tyr Val Arg Ile Arg Thr Thr
 1185 1190 1195 120
 Lys Asp Glu Glu Phe Ile Arg Lys Phe Ala Leu Phe Asp Glu Gln His
 1205 1210 1215

Arg Glu Glu Met Arg Lys Glu Arg Arg Arg Ile Gln Glu Gln Leu Arg
 1220 1225 1230
 Arg Leu Lys Arg Asn Gln Glu Lys Glu Lys Leu Lys Gly Pro Pro Glu
 1235 1240 1245
 Lys Lys Pro Lys Lys Met Lys Glu Arg Pro Asp Leu Lys Leu Lys Cys
 1250 1255 1260
 Gly Ala Cys Gly Ala Ile Gly His Met Arg Thr Asn Lys Phe Cys Pro
 1265 1270 1275 128
 Leu Tyr Tyr Gln Thr Asn Ala Pro Pro Ser Asn Pro Val Ala Met Thr
 1285 1290 1295
 Glu Glu Gln Glu Glu Glu Leu Glu Lys Thr Val Ile His Asn Asp Asn
 1300 1305 1310
 Glu Glu Leu Ile Lys Val Glu Gly Thr Lys Ile Val Leu Gly Lys Gln
 1315 1320 1325
 Leu Ile Glu Ser Ala Asp Glu Val Arg Arg Lys Ser Leu Val Leu Lys
 1330 1335 1340
 Phe Pro Lys Gln Gln Leu Pro Pro Lys Lys Lys Arg Arg Val Gly Thr
 1345 1350 1355 136
 Thr Val His Cys Asp Tyr Leu Asn Arg Pro His Lys Ser Ile His Arg
 1365 1370 1375
 Arg Arg Thr Asp Pro Met Val Thr Leu Ser Ser Ile Leu Glu Ser Ile
 1380 1385 1390
 Ile Asn Asp Met Arg Asp Leu Pro Asn Thr Tyr Pro Phe His Thr Pro
 1395 1400 1405
 Val Asn Ala Lys Val Val Lys Asp Tyr Tyr Lys Ile Ile Thr Arg Pro
 1410 1415 1420
 Met Asp Leu Gln Thr Leu Arg Glu Asn Val Arg Lys Arg Leu Tyr Pro
 1425 1430 1435 144
 Ser Arg Glu Glu Phe Arg Glu His Leu Glu Leu Ile Val Lys Asn Ser
 1445 1450 1455
 Ala Thr Tyr Asn Gly Pro Lys His Ser Leu Thr Gln Ile Ser Gln Ser
 1460 1465 1470
 Met Leu Asp Leu Cys Asp Glu Lys Leu Lys Glu Lys Glu Asp Lys Leu
 1475 1480 1485
 Ala Arg Leu Glu Lys Ala Ile Asn Pro Leu Leu Asp Asp Asp Gln
 1490 1495 1500
 Val Ala Phe Ser Phe Ile Leu Asp Asn Ile Val Thr Gln Lys Met Met
 1505 1510 1515 152
 Ala Val Pro Asp Ser Trp Pro Phe His His Pro Val Asn Lys Lys Phe
 1525 1530 1535
 Val Pro Asp Tyr Tyr Lys Val Ile Val Asn Pro Met Asp Leu Glu Thr
 1540 1545 1550
 Ile Arg Lys Asn Ile Ser Lys His Lys Tyr Gln Ser Arg Glu Ser Phe
 1555 1560 1565
 Leu Asp Asp Val Asn Leu Ile Leu Ala Asn Ser Val Lys Tyr Asn Gly
 1570 1575 1580
 Pro Glu Ser Gln Tyr Thr Lys Thr Ala Gln Glu Ile Val Asn Val Cys
 1585 1590 1595 160
 Tyr Gln Thr Leu Thr Glu Tyr Asp Glu His Leu Thr Gln Leu Glu Lys
 1605 1610 1615

Asp Ile Cys Thr Ala Lys Glu Ala Ala Leu Glu Glu Ala Glu Leu Glu
 1620 1625 1630
 Ser Leu Asp Pro Met Thr Pro Gly Pro Tyr Thr Pro Gln Pro Pro Asp
 1635 1640 1645
 Leu Tyr Asp Thr Asn Thr Ser Leu Ser Met Ser Arg Asp Ala Ser Val
 1650 1655 1660
 Phe Gln Asp Glu Ser Asn Met Ser Val Leu Asp Ile Pro Ser Ala Thr
 1665 1670 1675 168
 Pro Glu Lys Gln Val Thr Gln Glu Gly Glu Asp Gly Asp Gly Asp Leu
 1685 1690 1695
 Ala Asp Glu Glu Glu Gly Thr Val Gln Gln Pro Gln Ala Ser Val Leu
 1700 1705 1710
 Tyr Glu Asp Leu Leu Met Ser Glu Gly Glu Asp Asp Glu Glu Asp Ala
 1715 1720 1725
 Gly Ser Asp Glu Glu Gly Asp Asn Pro Phe Ser Ala Ile Gln Leu Ser
 1730 1735 1740
 Glu Ser Gly Ser Asp Ser Asp Val Gly Ser Gly Gly Ile Arg Pro Lys
 1745 1750 1755 176
 Gln Pro Arg Met Leu Gln Glu Asn Thr Arg Met Asp Met Glu Asn Glu
 1765 1770 1775
 Glu Ser Met Met Ser Tyr Glu Gly Asp Gly Gly Glu Ala Ser His Gly
 1780 1785 1790
 Leu Glu Asp Ser Asn Ile Ser Tyr Gly Ser Tyr Glu Glu Pro Asp Pro
 1795 1800 1805
 Lys Ser Asn Thr Gln Asp Thr Ser Phe Ser Ser Ile Gly Gly Tyr Glu
 1810 1815 1820
 Val Ser Glu Glu Glu Glu Asp Glu Glu Glu Glu Glu Gln Arg Ser Gly
 1825 1830 1835 184
 Pro Ser Val Leu Ser Gln Val His Leu Ser Glu Asp Glu Glu Asp Ser
 1845 1850 1855
 Glu Asp Phe His Ser Ile Ala Gly Asp Ser Asp Leu Asp Ser Asp Glu
 1860 1865 1870

<210> 7
 <211> 429
 <212> PRT
 <213> C. albicans

<400> 7

Glu Leu Leu Leu Asn Asn Pro Leu Asp Asn Ser Lys Gln Asn Arg Gln
 1 5 10 15
 Lys Ile Glu Asn Asp Asn Thr Thr Asn Asn Tyr Asn Gln Asn Ser
 20 25 30
 Asn Val Gln Asp Glu Glu Glu Asp Asp Ile Phe Asn Gly Gln Ile
 35 40 45
 Asn Leu Asp Lys Leu Lys Leu Asp Met Asn Asp Pro Asn Leu Leu Phe
 50 55 60
 Val Pro Ser Lys Lys Val Asp Ala Thr Lys Ser Val Val Pro Ser Thr
 65 70 75 80
 Asp Lys Leu Leu Glu Leu Lys Phe Asn Ile Ser Asn Asp Gln Glu Tyr

85 90 95
 Glu Leu Leu Arg Lys Asn Tyr Asn Thr Lys Gln Arg Ser Gln Leu Ser
 100 105 110
 Asn Leu Asn Ile Glu His Ser Val Pro Ala Leu Arg Leu Gln Thr Pro
 115 120 125
 Tyr Tyr Lys Val Lys Leu Ser Thr Asp Glu Thr Arg Ser Phe His Arg
 130 135 140
 Pro Val Phe Asn Val Arg Pro Gly Thr Leu Val Ser Phe Ser Lys Leu
 145 150 155 160
 Lys Leu Arg Lys Arg Lys Lys Asp Lys Gly Lys Ser Leu Gln Gln Ile
 165 170 175
 Phe Ser Lys Thr Ser Asp Leu Thr Val Ala Asp Thr Gly Asn Ile Ile
 180 185 190
 Ala Leu Glu Tyr Ser Glu Gln Tyr Pro Pro Ile Leu Ser Asn Phe Gly
 195 200 205
 Met Gly Ser Lys Leu Ile Asn Tyr Tyr Arg Lys Glu Arg Pro Asn Asp
 210 215 220
 Thr Ser Arg Pro Lys Ala Gln Ile Gly Glu Thr His Ile Leu Gly Val
 225 230 235 240
 Glu Asp Arg Ser Pro Phe Trp Asn Phe Gly Glu Val Ala Pro Gly Asp
 245 250 255
 Phe Val Pro Thr Leu Tyr Asn Asn Met Val Arg Ala Pro Ile Phe Lys
 260 265 270
 His Asp Asn Lys Pro Thr Asp Phe Leu Leu Val Lys Ser Gln Gly Ala
 275 280 285
 Gly Ser His Gln Lys Phe Tyr Leu Arg Gly Ile Asn Phe Asn Phe Ala
 290 295 300
 Val Gly Asn Thr Phe Pro Val Glu Val Pro Ala Pro His Ser Arg Lys
 305 310 315 320
 Val Thr Asn Ile Ser Lys Asn Arg Leu Lys Met Val Val Phe Arg Val
 325 330 335
 Met Asn Ser Leu Gly Val Pro Arg Ile Ser Val Lys Asp Val Ser Lys
 340 345 350
 His Phe Pro Glu His Ser Asp Met Gln Asn Arg Gln Arg Leu Lys Glu
 355 360 365
 Phe Met Glu Tyr Gln Arg Gln Gly Glu Asp Gln Gly Tyr Trp Lys Val
 370 375 380
 Arg Gly Leu Asn Asp Val Ile Pro Gly Glu Glu Glu Ile Arg Thr Met
 385 390 395 400
 Ile Thr Pro Glu Asp Ser Ser Leu Met Asp Thr Met Gln Phe Gly Gln
 405 410 415
 Gln Val Leu Asp Asp Asn Met Val Leu Phe Gly Glu Gln
 420 425

<210> 8
 <211> 434
 <212> PRT
 <213> S. cerevisiae

<400> 8

Met Thr Pro Asn Leu Lys Phe Ser Gly Gly Tyr Lys Leu Lys Ser Leu
 1 5 10 15
 Ile Glu Asp Val Ala Glu Asp Trp Gln Trp Asp Glu Asp Met Ile Ile
 20 25 30
 Asp Ala Lys Leu Lys Glu Ser Lys His Ala Glu Leu Asn Met Asn Asp
 35 40 45
 Glu Lys Leu Leu Leu Met Ile Glu Lys Thr Asn Asn Leu Ala Gln Gln
 50 55 60
 Lys Gln Gln Leu Asp Ser Ser Asn Leu Ile Leu Pro Leu Asn Glu Thr
 65 70 75 80
 Ile Leu Gln Gln Lys Phe Asn Leu Ser Asn Asp Asp Lys Tyr Gln Ile
 85 90 95
 Leu Lys Lys Thr His Gln Thr Lys Val Arg Ser Thr Ile Ser Asn Leu
 100 105 110
 Asn Ile Gln His Ser Gln Pro Ala Ile Asn Leu Gln Ser Pro Phe Tyr
 115 120 125
 Lys Val Ala Val Pro Arg Tyr Gln Leu Arg His Phe His Arg Glu Asn
 130 135 140
 Phe Gly Ser His Ile Arg Pro Gly Thr Lys Ile Val Phe Ser Lys Leu
 145 150 155 160
 Lys Ala Arg Lys Arg Lys Arg Asp Lys Gly Lys Asp Val Lys Glu Ser
 165 170 175
 Phe Ser Thr Ser Gln Asp Leu Thr Ile Gly Asp Thr Ala Pro Val Tyr
 180 185 190
 Leu Met Glu Tyr Ser Glu Gln Thr Pro Val Ala Leu Ser Lys Phe Gly
 195 200 205
 Met Ala Asn Lys Leu Ile Asn Tyr Tyr Arg Lys Ala Asn Glu Gln Asp
 210 215 220
 Thr Leu Arg Pro Lys Leu Pro Val Gly Glu Thr His Val Leu Gly Val
 225 230 235 240
 Gln Asp Lys Ser Pro Phe Trp Asn Phe Gly Phe Val Glu Pro Gly His
 245 250 255
 Ile Val Pro Thr Leu Tyr Asn Asn Met Ile Arg Ala Pro Val Phe Lys
 260 265 270
 His Asp Ile Ser Gly Thr Asp Phe Leu Leu Thr Lys Ser Ser Gly Phe
 275 280 285
 Gly Ile Ser Asn Arg Phe Tyr Leu Arg Asn Ile Asn His Leu Phe Thr
 290 295 300
 Val Gly Gln Thr Phe Pro Val Glu Glu Ile Pro Gly Pro Asn Ser Arg
 305 310 315 320
 Lys Val Thr Ser Met Lys Ala Thr Arg Leu Lys Met Ile Ile Tyr Arg
 325 330 335
 Ile Leu Asn His Asn His Ser Lys Ala Ile Ser Ile Asp Pro Ile Ala
 340 345 350
 Lys His Phe Pro Asp Gln Asp Tyr Gly Gln Asn Arg Gln Lys Val Lys
 355 360 365
 Glu Phe Met Lys Tyr Gln Arg Asp Gly Pro Glu Lys Gly Leu Trp Arg
 370 375 380
 Leu Lys Asp Asp Glu Lys Leu Leu Asp Asn Glu Ala Val Lys Ser Leu
 385 390 395 400

Ile Thr Pro Glu Gln Ile Ser Gln Val Glu Ser Met Ser Gln Gly Leu
 405 410 415
 Gln Phe Gln Glu Asp Asn Glu Ala Tyr Asn Phe Asp Ser Lys Leu Lys
 420 425 430
 Ser Leu

<210> 9
 <211> 415
 <212> PRT
 <213> S. pombe

<400> 9
 Val Asn Lys Thr Asn Gln Ser Ser Phe Phe Ile Asp Lys Ser Leu Val
 1 5 10 15
 Asp Ile Asp Phe Ala Phe Asp Glu Asn Ile Phe Asp Gly Asp Thr Gly
 20 25 30
 Thr Ser Lys Val Val Leu Asn Leu Asn Asp Pro Lys Leu Leu Leu Gln
 35 40 45
 Pro Gln Leu Pro Lys Lys Glu Asp Ser Gln Arg Ser Phe Ala Asp Thr
 50 55 60
 His Gln Arg Asn Ser Leu Ala Trp Lys Phe Asn Ile Ser Asn Asp Pro
 65 70 75 80
 Ala Tyr Glu Met Leu Lys Gln Asn His Gln Ser Lys Val Arg Asn Thr
 85 90 95
 Leu Ser Gln Leu Ala Ile Glu His Ala Ala Phe Ala Glu Lys Leu Thr
 100 105 110
 Phe Pro Tyr Tyr Lys Thr Arg Leu Ser Lys Arg Ala Val Arg Ser Tyr
 115 120 125
 His Arg Pro Thr Met Ser Phe Lys Pro Asn Ala Ala Ile Val Phe Ser
 130 135 140
 Pro Leu Ile Val Arg Lys Arg Ser Lys Asp Lys His Lys Ser Glu Arg
 145 150 155 160
 Glu Leu Ile Pro Thr Thr Lys Glu Ile Thr Met Gly Asp Thr Thr His
 165 170 175
 Ala Ile Leu Val Glu Phe Ser Glu Glu His Pro Ala Val Leu Ser Asn
 180 185 190
 Ala Gly Met Ala Ser Arg Ile Val Asn Tyr Tyr Arg Lys Lys Asn Glu
 195 200 205
 Gln Asp Glu Ser Arg Pro Lys Leu Glu Val Gly Glu Ser His Val Leu
 210 215 220
 Asp Val Gln Asp Arg Ser Pro Phe Trp Asn Phe Gly Ser Val Glu Pro
 225 230 235 240
 Gly Glu Ile Thr Pro Thr Leu Tyr Asn Lys Met Ile Arg Ala Pro Leu
 245 250 255
 Phe Lys His Glu Val Pro Pro Thr Asp Phe Ile Leu Ile Arg Asn Ser
 260 265 270
 Ser Ser Tyr Gly Ser Lys Tyr Tyr Leu Lys Asn Ile Asn His Met Phe
 275 280 285
 Val Ser Gly Gln Thr Phe Pro Val Thr Asp Val Pro Gly Pro His Ser

290 295 300
 Arg Lys Val Thr Thr Ala Ser Lys Asn Arg Leu Lys Met Leu Val Phe
 305 310 315 320
 Arg Leu Ile Arg Arg Ser Pro Asn Gly Gly Leu Phe Ile Arg Gln Leu
 325 330 335
 Ser Lys His Phe Ser Asp Gln Asn Glu Met Gln Ile Arg Gln Arg Leu
 340 345 350
 Lys Glu Phe Met Glu Tyr Lys Lys Lys Gly Asp Gly Pro Gly Tyr Trp
 355 360 365
 Lys Leu Lys Ser Asn Glu Val Val Pro Asp Glu Ala Gly Thr Arg Ser
 370 375 380
 Met Val Ser Pro Glu Thr Val Cys Leu Leu Glu Ser Met Gln Val Gly
 385 390 395 400
 Val Arg Gln Leu Glu Asp Ala Gly Tyr Gly Lys Thr Met Asp Glu
 405 410 415

<210> 10
 <211> 481
 <212> PRT
 <213> Human

<400> 10
 Ser Leu Ala Gly Trp Leu Pro Ser Ser Met Thr Arg Asn Ala Met Ala
 1 5 10 15
 Tyr Asn Val Gln Gln Gly Phe Ala Ala Thr Leu Asp Asp Asp Lys Pro
 20 25 30
 Trp Tyr Ser Ile Phe Pro Ile Asp Asn Glu Asp Leu Val Tyr Gly Arg
 35 40 45
 Trp Glu Asp Asn Ile Ile Trp Asp Ala Gln Ala Met Pro Arg Leu Leu
 50 55 60
 Glu Pro Pro Val Leu Thr Leu Asp Pro Asn Asp Glu Asn Leu Ile Leu
 65 70 75 80
 Glu Ile Pro Asp Glu Lys Glu Glu Ala Thr Ser Asn Ser Pro Ser Lys
 85 90 95
 Glu Ser Lys Lys Glu Ser Ser Leu Lys Lys Ser Arg Ile Leu Leu Gly
 100 105 110
 Lys Thr Gly Val Ile Lys Glu Glu Pro Gln Gln Asn Met Ser Gln Pro
 115 120 125
 Glu Val Lys Asp Pro Trp Asn Leu Ser Asn Asp Glu Tyr Tyr Tyr Pro
 130 135 140
 Lys Gln Gln Gly Leu Arg Gly Thr Phe Gly Gly Asn Ile Ile Gln His
 145 150 155 160
 Ser Ile Pro Ala Val Glu Leu Arg Gln Pro Phe Phe Pro Thr His Met
 165 170 175
 Gly Pro Ile Lys Leu Arg Gln Phe His Arg Pro Pro Leu Lys Lys Tyr
 180 185 190
 Ser Phe Gly Ala Leu Ser Gln Pro Gly Pro His Ser Val Gln Pro Leu
 195 200 205
 Leu Lys His Ile Lys Lys Lys Ala Lys Met Arg Glu Gln Glu Arg Gln
 210 215 220

Ala Ser Gly Gly Gly Glu Met Phe Phe Met Arg Thr Pro Gln Asp Leu
 225 230 235 240
 Thr Gly Lys Asp Gly Asp Leu Ile Leu Ala Glu Tyr Ser Glu Glu Asn
 245 250 255
 Gly Pro Leu Met Met Gln Val Gly Met Ala Thr Lys Ile Lys Asn Tyr
 260 265 270
 Tyr Lys Arg Lys Pro Gly Lys Asp Pro Gly Ala Pro Asp Cys Lys Tyr
 275 280 285
 Gly Glu Thr Val Tyr Cys His Thr Ser Pro Phe Leu Gly Ser Leu His
 290 295 300
 Pro Gly Gln Leu Leu Gln Ala Phe Glu Asn Asn Leu Phe Arg Ala Pro
 305 310 315 320
 Ile Tyr Leu His Lys Met Pro Glu Thr Asp Phe Leu Ile Ile Arg Thr
 325 330 335
 Arg Gln Gly Tyr Tyr Ile Arg Glu Leu Val Asp Ile Phe Val Val Gly
 340 345 350
 Gln Gln Cys Pro Leu Phe Glu Val Pro Gly Pro Asn Ser Lys Arg Ala
 355 360 365
 Asn Thr His Ile Arg Asp Phe Leu Gln Val Phe Ile Tyr Arg Leu Phe
 370 375 380
 Trp Lys Ser Lys Asp Arg Pro Arg Arg Ile Arg Met Glu Asp Ile Lys
 385 390 395 400
 Lys Ala Phe Pro Ser His Ser Glu Ser Ser Ile Arg Lys Arg Leu Lys
 405 410 415
 Leu Cys Ala Asp Phe Lys Arg Thr Gly Met Asp Ser Asn Trp Trp Val
 420 425 430
 Leu Lys Ser Asp Phe Arg Leu Pro Thr Glu Glu Glu Ile Arg Ala Met
 435 440 445
 Val Ser Pro Glu Gln Cys Cys Ala Tyr Tyr Ser Met Ile Ala Ala Glu
 450 455 460
 Gln Arg Leu Lys Asp Ala Gly Tyr Gly Glu Lys Ser Phe Phe Ala Pro
 465 470 475 480
 Glu

<210> 11
 <211> 74
 <212> PRT
 <213> C. albicans

<400> 11
 Asp Ala Glu Asn Gly Asp Asp Ile Asn Lys Asp Lys Glu Lys Glu Val
 1 5 10 15
 Glu Lys Glu Lys Glu Gln Glu Arg Glu Glu Glu Lys Gly Lys Asp Lys
 20 25 30
 Glu Lys Asp Lys Asp Lys Glu Lys Asp Lys Thr Glu Lys Glu Lys Ser
 35 40 45
 Lys Lys Ser Lys Glu Gln Asp Thr Glu Ile Asp Val Glu Glu Glu Leu
 50 55 60
 Ala Pro Trp Asn Leu Ser Arg Asn Phe Val

65

70

<210> 12
<211> 18
<212> DNA
<213> "Artificial Sequence"

<220>

<223> Inosine

<400> 12
ccwggwccwa aytcnadd 18

<210> 13
<211> 23
<212> DNA
<213> "Artificial Sequence"

<400> 13
gayccwachg gwtgtggwga agg 23

<210> 14
<211> 24
<212> DNA
<213> "Artificial Sequence"

<400> 14
cctttcwcca cawccagtwg grtc 24

<210> 15
<211> 19
<212> DNA
<213> "Artificial Sequence"

<220>

<223> Inosine

<400> 15
ttrtthcayc tnartgwcc 19

<210> 16
<211> 30
<212> DNA
<213> "Artificial Sequence"

<400> 16
ccgctcgaga tgacacccaa cttaaagtcc 30

<210> 17
 <211> 29
 <212> DNA
 <213> "Artificial Sequence"

<400> 17
 cgcggatcca gagatttttag cttagaatc 29

<210> 18
 <211> 37
 <212> DNA
 <213> "Artificial Sequence"

<400> 18
 ggaattccat atgcttttgc tcaacaatcc cttggac 37

<210> 19
 <211> 32
 <212> DNA
 <213> "Artificial Sequence"

<400> 19
 cgcggatccc tgctctgctc accgaataac ac 32

<210> 20
 <211> 37
 <212> DNA
 <213> "Artificial Sequence"

<400> 20
 ggaattccat atgagcctgg caggctggct tccttct 37

<210> 21
 <211> 33
 <212> DNA
 <213> "Artificial Sequence"

<400> 21
 ccgctcgagt tctggagcaa aaaaggattt ctc 33

<210> 22
 <211> 0
 <212> DNA
 <213> Human

<400> 22
 Met Gly Pro Gly Cys Asp Leu Leu Leu Arg Thr Ala Ala Thr Ile Thr
 1 5 10 15
 Ala Ala Ala Ile Met Ser Asp Thr Asp Ser Asp Glu Asp Ser Ala Gly
 20 25 30

Gly Gly Pro Phe Ser Leu Ala Gly Phe Leu Phe Gly Asn Ile Asn Gly
 35 40 45
 Ala Gly Gln Leu Glu Gly Glu Ser Val Leu Asp Asp Glu Cys Lys Lys
 50 55 60
 His Leu Ala Gly Leu Gly Ala Leu Gly Leu Gly Ser Leu Ile Thr Glu
 65 70 75 80
 Leu Thr Ala Asn Glu Glu Leu Thr Gly Thr Asp Gly Ala Leu Val Asn
 85 90 95
 Asp Glu Gly Trp Val Arg Ser Thr Glu Asp Ala Val Asp Tyr Ser Asp
 100 105 110
 Ile Asn Glu Val Ala Glu Asp Glu Ser Arg Arg Tyr Gln Gln Thr Met
 115 120 125
 Gly Ser Leu Gln Pro Leu Cys His Ser Asp Tyr Asp Glu Asp Asp Tyr
 130 135 140
 Asp Ala Asp Cys Glu Asp Ile Asp Cys Lys Leu Met Pro Pro Pro Pro
 145 150 155 160
 Pro Pro Pro Gly Pro Met Lys Lys Asp Lys Asp Gln Asp Ser Ile Thr
 165 170 175
 Gly Glu Lys Val Asp Phe Ser Ser Ser Ser Asp Ser Glu Ser Glu Met
 180 185 190
 Gly Pro Gln Glu Ala Thr Gln Ala Glu Ser Glu Asp Gly Lys Leu Thr
 195 200 205
 Leu Pro Leu Ala Gly Ile Met Gln His Asp Ala Thr Lys Leu Leu Pro
 210 215 220
 Ser Val Thr Glu Leu Phe Pro Glu Phe Arg Pro Gly Lys Val Leu Arg
 225 230 235 240
 Phe Leu Arg Leu Phe Gly Pro Gly Lys Asn Val Pro Ser Val Trp Arg
 245 250 255
 Ser Ala Arg Arg Lys Arg Lys Lys Lys His Arg Glu Leu Ile Gln Glu
 260 265 270
 Glu Gln Ile Gln Glu Val Glu Cys Ser Val Glu Ser Glu Val Ser Gln
 275 280 285
 Lys Ser Leu Trp Asn Tyr Asp Tyr Ala Pro Pro Pro Pro Pro Glu Gln
 290 295 300
 Cys Leu Ser Asp Asp Glu Ile Thr Met Met Ala Pro Val Glu Ser Lys
 305 310 315 320
 Phe Ser Gln Ser Thr Gly Asp Ile Asp Lys Val Thr Asp Thr Lys Pro
 325 330 335
 Arg Val Ala Glu Trp Arg Tyr Gly Pro Ala Arg Leu Trp Tyr Asp Met
 340 345 350
 Leu Gly Val Pro Glu Asp Gly Ser Gly Phe Asp Tyr Gly Phe Lys Leu
 355 360 365
 Arg Lys Thr Glu His Glu Pro Val Ile Lys Ser Arg Met Ile Glu Glu
 370 375 380
 Phe Arg Lys Leu Glu Glu Asn Asn Gly Thr Asp Leu Leu Ala Asp Glu
 385 390 395 400
 Asn Phe Leu Met Val Thr Gln Leu His Trp Glu Asp Asp Ile Ile Trp
 405 410 415
 Asp Gly Glu Asp Val Lys His Lys Gly Thr Lys Pro Gln Arg Ala Ser
 420 425 430

Leu Ala Gly Trp Leu Pro Ser Ser Met Thr Arg Asn Ala Met Ala Tyr
 435 440 445
 Asn Val Gln Gln Gly Phe Ala Ala Thr Leu Asp Asp Asp Lys Pro Trp
 450 455 460
 Tyr Ser Ile Phe Pro Ile Asp Asn Glu Asp Leu Val Tyr Gly Arg Trp
 465 470 475 480
 Glu Asp Asn Ile Ile Trp Asp Ala Gln Ala Met Pro Arg Leu Leu Glu
 485 490 495
 Pro Pro Val Leu Thr Leu Asp Pro Asn Asp Glu Asn Leu Ile Leu Glu
 500 505 510
 Ile Pro Asp Glu Lys Glu Glu Ala Thr Ser Asn Ser Pro Ser Lys Glu
 515 520 525
 Ser Lys Lys Glu Ser Ser Leu Lys Lys Ser Arg Ile Leu Leu Gly Lys
 530 535 540
 Thr Gly Val Ile Lys Glu Glu Pro Gln Gln Asn Met Ser Gln Pro Glu
 545 550 555 560
 Val Lys Asp Pro Trp Asn Leu Ser Asn Asp Glu Tyr Tyr Tyr Pro Lys
 565 570 575
 Gln Gln Gly Leu Arg Gly Thr Phe Gly Gly Asn Ile Ile Gln His Ser
 580 585 590
 Ile Pro Ala Val Glu Leu Arg Gln Pro Phe Phe Pro Thr His Met Gly
 595 600 605
 Pro Ile Lys Leu Arg Gln Phe His Arg Pro Pro Leu Lys Lys Tyr Ser
 610 615 620
 Phe Gly Ala Leu Ser Gln Pro Gly Pro His Ser Val Gln Pro Leu Leu
 625 630 635 640
 Lys His Ile Lys Lys Lys Ala Lys Met Arg Glu Gln Glu Arg Gln Ala
 645 650 655
 Ser Gly Gly Gly Glu Met Phe Phe Met Arg Thr Pro Gln Asp Leu Thr
 660 665 670
 Gly Lys Asp Gly Asp Leu Ile Leu Ala Glu Tyr Ser Glu Glu Asn Gly
 675 680 685
 Pro Leu Met Met Gln Val Gly Met Ala Thr Lys Ile Lys Asn Tyr Tyr
 690 695 700
 Lys Arg Lys Pro Gly Lys Asp Pro Gly Ala Pro Asp Cys Lys Tyr Gly
 705 710 715 720
 Glu Thr Val Tyr Cys His Thr Ser Pro Phe Leu Gly Ser Leu His Pro
 725 730 735
 Gly Gln Leu Leu Gln Ala Phe Glu Asn Asn Leu Phe Arg Ala Pro Ile
 740 745 750
 Tyr Leu His Lys Met Pro Glu Thr Asp Phe Leu Ile Ile Arg Thr Arg
 755 760 765
 Gln Gly Tyr Tyr Ile Arg Glu Leu Val Asp Ile Phe Val Val Gly Gln
 770 775 780
 Gln Cys Pro Leu Phe Glu Val Pro Gly Pro Asn Ser Lys Arg Ala Asn
 785 790 795 800
 Thr His Ile Arg Asp Phe Leu Gln Val Phe Ile Tyr Arg Leu Phe Trp
 805 810 815
 Lys Ser Lys Asp Arg Pro Arg Arg Ile Arg Met Glu Asp Ile Lys Lys
 820 825 830

Ala Phe Pro Ser His Ser Glu Ser Ser Ile Arg Lys Arg Leu Lys Leu
 835 840 845
 Cys Ala Asp Phe Lys Arg Thr Gly Met Asp Ser Asn Trp Trp Val Leu
 850 855 860
 Lys Ser Asp Phe Arg Leu Pro Thr Glu Glu Glu Ile Arg Ala Met Val
 865 870 875 880
 Ser Pro Glu Gln Cys Cys Ala Tyr Tyr Ser Met Ile Ala Ala Glu Gln
 885 890 895
 Arg Leu Lys Asp Ala Gly Tyr Gly Glu Lys Ser Phe Phe Ala Pro Glu
 900 905 910
 Glu Glu Asn Glu Glu Asp Phe Gln Met Lys Ile Asp Asp Glu Val Arg
 915 920 925
 Thr Ala Pro Trp Asn Thr Thr Arg Ala Phe Ile Ala Ala Met Lys Gly
 930 935 940
 Lys Cys Leu Leu Glu Val Thr Gly Val Ala Asp Pro Thr Gly Cys Gly
 945 950 955 960
 Glu Gly Phe Ser Tyr Val Lys Ile Pro Asn Lys Pro Thr Gln Gln Lys
 965 970 975
 Asp Asp Lys Glu Pro Gln Pro Val Lys Lys Thr Val Thr Gly Thr Asp
 980 985 990
 Ala Asp Leu Arg Arg Leu Ser Leu Lys Asn Ala Lys Gln Leu Leu Arg
 995 1000 1005
 Lys Phe Gly Val Pro Glu Glu Glu Ile Lys Lys Leu Ser Arg Trp Glu
 1010 1015 1020
 Val Ile Asp Val Val Arg Thr Met Ser Thr Glu Gln Ala Arg Ser Gly
 1025 1030 1035 104
 Glu Gly Pro Met Ser Lys Phe Ala Arg Gly Ser Arg Phe Ser Val Ala
 1045 1050 1055
 Glu His Gln Glu Arg Tyr Lys Glu Glu Cys Gln Arg Ile Phe Asp Leu
 1060 1065 1070
 Gln Asn Lys Val Leu Ser Ser Thr Glu Val Leu Ser Thr Asp Thr Asp
 1075 1080 1085
 Ser Ser Ser Ala Glu Asp Ser Asp Phe Glu Glu Met Gly Lys Asn Ile
 1090 1095 1100
 Glu Asn Met Leu Gln Asn Lys Lys Thr Ser Ser Gln Leu Ser Arg Glu
 1105 1110 1115 112
 Arg Glu Glu Gln Glu Arg Lys Glu Leu Gln Arg Met Leu Leu Ala Ala
 1125 1130 1135
 Gly Ser Ala Ala Ser Gly Asn Asn His Arg Asp Asp Asp Thr Ala Ser
 1140 1145 1150
 Val Thr Ser Leu Asn Ser Ser Ala Thr Gly Arg Cys Leu Lys Ile Tyr
 1155 1160 1165
 Arg Thr Phe Arg Asp Glu Glu Gly Lys Glu Tyr Val Arg Cys Glu Thr
 1170 1175 1180
 Val Arg Lys Pro Ala Val Ile Asp Ala Tyr Val Arg Ile Arg Thr Thr
 1185 1190 1195 120
 Lys Asp Glu Glu Phe Ile Arg Lys Phe Ala Leu Phe Asp Glu Gln His
 1205 1210 1215
 Arg Glu Glu Met Arg Lys Glu Arg Arg Arg Ile Gln Glu Gln Leu Arg
 1220 1225 1230

Arg Leu

<210> 23
 <211> 52
 <212> DNA
 <213> C. albicans

<400> 23
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<210> 27
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<400> 27
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<210> 28
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